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(54) Title: METHOD FOR EARLY DIAGNOSIS OF, AND DETERMINATION OF PROGNOSIS IN, CANCER

(57) Abstract

The invention provides a method for diagnosis of, and determining a prognosis for, cancer causatively associated with derangements of chromosome 9p21. Underlying the invention is the discovery that such derangements have their genesis in deletions occurring centromeric to STS 3.21, most often including breakpoints in exon 8 and/or between exons 4 and 5 of the gene which codes for methylthioadenosine phosphorylase. As the cancer and tumor development advance, deletions in 9p21 progress centromerically from the genesis point toward the gene encoding p16. Thus, the method of the invention is performed by determining whether (a) portions of the 9p21 region including and telomeric to STS 3.21 are deleted; and (b) portions of the 9p21 region centromeric to STS 3.21 are deleted; wherein a positive finding in step (a) and a negative finding in step (b) are indicative of a cancer in an early stage of tumor development and a positive finding in step (b) is indicative of a cancer in an advanced stage of tumor development.

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METHOD FOR EARLY DIAGNOSIS OF, AND DETERMINATION OF PROGNOSIS IN, CANCER

STATEMENT REGARDING RELATED APPLICATIONS

This application is a utility conversion of U.S. Provisional Patent Application Serial No. 60/090411, filed on June 23, 1998, the priority of which is claimed.

STATEMENT REGARDING GOVERNMENT SUPPORT

The work underlying this invention was supported by a grant from the National Institutes of Health, Grant Numbers 5 UO1 CA64976 and 5 R21 CA68260. The Government may have certain rights in the invention.

BACKGROUND OF THE INVENTION

1. Field of the Invention

The invention relates to methods for early diagnosis and staging of cancer which is causatively related to derangements of chromosomal 9p2l in mammals.

2. History of the Related Art

The chromosomal region 9p2l harbors five different genes within about 120 kb: the tumor suppressor genes pl5IINK4B (CDKN4B, hereafter "p15") with its alternative spliced form pl0, pl6INK4A (CDKN2A, a key regulator of the G1-phase of the cell cycle and growth control, hereafter "p 16") and pl9ARF (which acts via a p53 dependent pathway to block cell cycle progression), as well as the gene for the metabolic enzyme methylthioadenosine phosphorylase (MTAP).

Homozygous deletion is the most important mechanism of inactivation of all three genes. While somatic and germline mutations have been described for p16, they

seem to be very rare in the other two genes. A third mechanism of inactivation is hypermethylation of CpG-islands in the promoter region of both p15 and p16, which results in silencing of transcription. No relationship between hypermethylation and tumor grade has been observed.

Studies of tumor cell lines and gross primary tumors reveal that homozygous deletions of p16 are found in 10-60 % of brain tumors, depending on the tumor stage and histology. Homozygous deletions of p16 are also present in melanomas, lung cancers, malignant mesotheliomas, bladder cancers, pancreatic carcinomas, ovarian carcinomas, head and neck cancers, chondrosarcomas, esophageal squamous cell carcinomas, T-cell acute lymphoblastic leukemias, and other primary lymphoid malignancies.

Loss of heterozygosity (LOH) as well as hemi- and homozygous deletions of the 9p2l region encompassing the pl61NKA4 gene and MTAP genes have been described in a variety of human tumors, primarily based on analysis of tumor cell lines, including acute lymphoblastic leukemia (ALL), melanoma, ovarian cancer, glioma, head and neck cancer, bladder cancer, chondrosarcoma, small cell and non-small cell lung cancer (NSCLC). In general, it has been widely concluded that the portion of 9p2l where the p16 INK4A gene resides is the point at which derangements in the region of 9p2l between that and the MTAP gene begin, indicating that p16 would be a marker for cancer at early and more advanced stages of tumor development.

SUMMARY OF THE INVENTION

The invention provides a method for differentiating among stages of tumor development which is causatively related to derangements of chromosomal region 9p2l in mammals. More particularly, the invention provides a framework for both early diagnosis of such cancers and prognosis based on the stage of tumor development. In the paradigm provided by the invention, derangements of 9p2l in many cancers begin at the MTAP gene locus at the onset of tumor development and

progress centromerically toward the p16 gene locus as tumor development advances, as represented in the pictorial below:

Chromosome 9p21*

						Centromeric
p15*	STS 5BS	p16*	STS 54F	STS 2F	STS 3.21	MTAP*
††	z	†††	z	z	z	††††††††
12		123				87654321

†: indicates the location of an exon.

*References:

NUCLEOTIDE	REPRESENTATIVE CITATIONS FOR NUCLEOTIDE SEQUENCE
9p21	ATCC** AC00047 (updating AC00049 and AC00048); FIG. 7; SEQ.ID.No.: 25
p15	ATCC S75756
p16	ATCC S69822; ATCC S69804; AF 044170
MTAP	ATCC NM002451.1 (updating ATCC U22233); FIG. 8; SEQ.ID.No.: 26

**ATCC=American Type Culture Collection

According to the method of the invention, a model pattern of deletion of portions of chromosome 9p21 ("staging reference"; see, e.g., Fig. 1) is used as a reference for comparison to 9p21 in samples of cells which are suspected of, or confirmed as, being cancerous. The sample cells are analyzed for the presence of deletions and breakpoints in 9p21. The deletion pattern identified in the sample cells is compared against the staging reference, in which the presence or absence of certain deletions and breakpoints in 9p21 are correlated to the stage of development of cancer in the sample cell population.

The data upon which the staging reference is based are derived from analysis of 9p21 in histological grade, paraffin-embedded samples of human tumors at various stages of tumor development identified according to conventional staging techniques. Unlike the tumor cell lines used in previous analyses of 9p21 derangements, the

unique tissue samples utilized as the basis for development of the staging reference include very early stage tumors as well as advanced stage tumors.

Surprisingly, analysis of the tissue samples revealed that, in contrast to widely held perception, the pl6INK4a (p16) gene is not the starting point for the 9p2l derangements observed in cancer cell lines and advanced primary tumors. Rather, the derangement starting point in many tumor cells lies just centromeric to exon 8 of the MTAP gene (telomeric to sequence tagged site [STS] 3.21), while in others the first breakpoint is within the MTAP gene, between exons 4 and 5. As tumor development progresses, deletions within 9p21 advance centromerically toward inclusion of all or part of the pl6 coding sequence, and may also advance telomerically toward of all or part of the MTAP coding sequence. An additional fragile site lying between p16 and p15INK4b (p15) can lead to deletion of a portion of 9p21 in more advanced tumor cells. Thus, pl6 deletion alone is not a reliable marker for either early diagnosis or prognostic staging of cancer, both of which can be accomplished by use of the staging reference of the invention.

The invention also provides methods and reagents for use in early diagnosis of cancer through hybridization to early deletion points in 9p2l.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE 1 is a graphical representation of the staging reference of the invention, wherein the dotted line indicates the progression of derangements in 9p21, solid circles (•) indicate the absence of a deletion (normal gene structure) and open circles (O) indicate the occurrence of a deletion. The figure uses the breakpoint in MTAP gene exon 8 as the start point for 9p21 derangement and therefore represents (determined as described in the Detailed Description) a common paradigm in early cancer development.

FIGURE 2 is a graphic table which identifies the location of the earliest detectable homozygous deletions of a region of chromosome 9p2l in histological grade human primary tumor cell samples obtained as described in the Detailed Description. The identity of the tumor cell type and its histological grade is shown in the leftmost column of the table. Solid circles (•) in the remaining columns denote the absence of a deletion in the indicated region of 9p2l (normal gene structure) while an open circle (O) denotes a deletion in the region.

FIGURE 3 is a chart which relates the occurrence of a homozygous deletion occurring in the MTAP gene with or without involvement of the p16 coding sequence, as well as normal 9p2l spanning the sites of these genes, with the stage of cancer in tested tumor samples obtained as described in the Detailed Description. In the Figure legend, (+) refers to the absence of a deletion in the indicated region of 9p2l (normal gene structure) and (-) refers to the occurrence of a deletion in the region. Cancer grade is indicated along the bottom axis of the Figure.

FIGURE 4 is a graphic table which identifies the location of the earliest detectable homozygous deletions of a region of chromosome 9p2l in xenograft tumor cell samples obtained as described in the Detailed Description. The identity of the tumor tissue type is shown in the leftmost column of the table. Solid circles (?) in the remaining columns denote the absence of a deletion in the indicated region of 9p2l (normal gene structure) while an open circle (?) denotes a deletion in the region. The xenograft samples involving adult tumors are the 11 samples listed in the bottom half of the leftmost column, while samples involving childhood tumors are the 5 samples

listed in the upper half of the leftmost column.

FIGURE 5(a) is a blot radiograph depicting p16 expression in xenograft brain tumors while Figure 5(b) is a blot radiograph depicting MTAP expression in xenograft brain tumors.

FIGURE 6 is a graph correlating MTAP/control gene (MTAPΨ) and p16/control gene dosage rations in adenosarcomas (?) and in squamous and large cell carcinomas (O).

FIGURE 7 is a cosmid nucleotide sequence including 9p21 published in the GENBANK? DNA Sequence database, with reference to ATCC AC00047.

FIGURE 8 is a genomic nucleotide sequence for the MTAP gene, with the exons highlighted by underlining.

DETAILED DESCRIPTION OF THE INVENTION

The invention provides a method for diagnosis of, and determining a prognosis for, cancer causatively associated with derangements of chromosome 9p21. Underlying the invention is the discovery that such derangements have their genesis in deletions occurring centromeric to STS 3.21, most often including breakpoints in exon 8 and/or between exons 4 and 5 of the gene which codes for methylthioadenosine phosphorylase (MTAP).

As the cancer and tumor development advance, deletions in 9p21 progress centromerically from the genesis point toward the gene encoding p16. Thus, the method of the invention is performed by determining whether (a) portions of the 9p21 region including and telomeric to STS 3.21 are deleted; and (b) portions of the 9p21 region centromeric to STS 3.21 are deleted; wherein a positive finding in step (a) and a negative finding in step (b) are indicative of a cancer in an early stage of tumor development and a positive finding in step (b) is indicative of a cancer in an advanced stage of tumor development.

In this respect, the phrase "an early stage of tumor development" refers to tumors of histologic grade III or lower, with the majority falling within histologic grades I and II. Furthermore, the phrase "an advanced stage of tumor development" refers to tumors of histologic grade III or higher, with the majority falling within histologic grade IV. In the invention, histologic grade III is considered as a portal grade, a finding for which is indicative of progressive tumor development from an early to an advanced stage. Histologic evaluation of a tumor may be utilized to determine the exact grade into which a particular tumor whose stage is identified according to the method of the invention falls.

The data described herein are derived from investigation into the scope of 9p21 deletions in 95 brain tumors of different stages (of both astrocytic and oligodendroglial origin), as well as in non-small cell lung cancer (NSCLC). These types of cancer are representative of those associated with derangements in 9p21.

Of the brain tumors, only eleven glioblastomas had p16 deletions without MTAP deletions. Unexpectedly, eight out of 95 tumors (8%) showed homozygous

MTAP deletions without p16 deletions and these comprised 23% of the histologic grade I-III specimens. In fourteen cases, including 12 glioblastomas, both p16 and MTAP were deleted. No grade I-III tumor had an isolated p16 deletion, establishing that the p16 gene is not the genesis of 9p21 derangements in these cancers.

Of the NSCLC samples (including 25 adenocarcinomas (50%), 21 squamous cell carcinomas (42%) and 4 large cell carcinomas (8%)), homozygous deletions of MTAP exon 8 could be detected in 19 of 50 NSCLC samples (38%). Adenocarcinoma (11 of 25, 44%) showed a higher deletion frequency than squamous cell carcinoma (6 of 21, 29%). In contrast, homozygous p16 deletions were detected in only 9 of 50 (18%) samples using specific primers for p16 exon 1. No difference between the histological subtypes and p16 deletion frequency was observed. Interestingly, among the 10 samples with MTAP deletions but intact p16 exon 1 (evaluated with primers specific for p16 exon 3, the exon nearest to MTAP exon 8; see Table 1 below), none of the samples had a deletion of the p16 exon 3 coding region.

To localize the deletion breakpoints which lead to the inactivation of the genes, the region between p15 and MTAP was mapped using specific primers for recently defined sequence tagged sites (STS) as well as for the different exons of the genes. In summary, common breakpoints within the MTAP gene occurred between exons 4 and 5 and between exons 7 and 8. Similar breakpoints were observed in brain tumor xenografts (5 childhood and 11 adult brain tumors) propagated in nude mice, although deletions of p16 and MTAP in childhood tumors were rare in comparison to the occurrence of p15 deletions.

Western blot analysis showed that the MTAP protein was present in all tested tumors that contained the MTAP gene, whereas two tumors with an intact p16 gene lacked detectable p16 protein. Xenografts with breakpoints between MTAP exons 7/8 and exons 4/5 produced immunoreactive MTAP proteins of about 32 kDa and 28 kDa, respectively, and MTAP mRNA. These results demonstrate that homozygous MTAP without p16 deletions are common in low-grade gliomas.

The staging reference model (Fig. 1) for cancer progression from a deletion at the breakpoint at MTAP gene exon 8 represents a common paradigm for early cancer

development in gliomas and other cancers associated with 9p21 chromosomal derangements. Among adult tumors, deletions at breakpoints in MTAP exon 8 and/or between MTAP exons 4 and 5, without involvement of 9p21 centromeric to MTAP exon 8, exclusively occur in low-grade tumors (e.g., stage III and below in gliomas). Detection of this phenotype in a tumor therefore evidences early cancer development and, presumably, greater susceptibility to treatment. In contrast, derangements of 9p21 involving deletions of the MTAP gene and regions centromeric thereto, including the p16 gene, evidence advanced tumor development (mirroring the condition present in tumor cell lines). In contrast, MTAP/p16 combination deletions are relatively rare and are preceded by the more predominant deletion of p15.

An important result of the deletion analysis in brain tumors was that twenty-three percent of the grade II-III gliomas were MTAP negative and p16 positive, compared to only 2% of the grade IV tumors (p=0.0005 by chi square analysis). The single glioblastoma with an isolated MTAP deletion had a p16 gene dosage level closest to the negative cutoff point, among all the tumors analyzed. Moreover, there was a positive correlation between the size of the deletions and the grade of the tumor, with large deletions involving p16 predominantly confined to glioblastomas.

Twenty-three out of the 25 p16 deletions were in grade IV tumors. This is in accordance with previous findings. Despite differences in the exact percentages of p16 deletions detected in different studies, it seems clear that homozygous deletion of this cdk-inhibitor is a feature of high-grade gliomas. In addition, p16 is more often deleted in astrocytic than in non-astrocytic tumors.

Although the invention is not limited by any theory concerning the genetic causes for the findings described herein, the staging reference (exemplified in Fig. 1) could explain the evolution of glioblastomas in the adult. According to this scheme, an initial, small deletion occurs close to MTAP exon 8, and inactivates a functional motif, such as a new gene, another INK4 exon, or a promoter/enhancer site for p16 or p19ARF. This deletion may cause genetic instability in the 9p21 region, leading to the loss of additional chromosomal material during further cell doublings. The new breakpoints would tend to occur at fragile sites, which explains the presence of

distinct breakpoints, e.g., between MTAP exons 4 and 5, and between p15 and p16.

With respect to childhood cancers, the expression of p16 increases as fibroblasts approach senescence. On the other hand, neither p16 nor pl9ARF is highly expressed in embryonic tissues, whereas p15 (as well as the INK4 genes pl8INK4c and pl9INK4d) expression has been detected in fetal tissues of mouse and man. The p16 gene locus was intact in 4 out of 5 of childhood glioma xenografts studied, whereas the p15 locus was deleted in all but one of the tumors (Fig. 3). These preliminary results are consistent with the concept that p15, rather than p16, plays a role in early organogenesis of the brain and that p15 inactivation is a frequent step in the development of childhood gliomas.

These data were developed as described further below. The staging model set forth in Fig. 1 provides a common paradigm for the brain tumors studies and relates 9p2l derangements and their progression from exon 8 of the MTAP gene to clinical grades of tumors. Detection of derangements in 9p2l matched to the staging reference reveal both the probable presence of a tumor (even, significantly, at a stage of development not detectable on gross examination) and its probable grade of advancement. Those of ordinary skill in the art will be familiar with the clinical significance from a prognostic viewpoint of determining the grade of particular cancers, as set forth in, for example, Ginsberg, *Oncology*, 12(1 Suppl. *2*):51-54 (1998) [NSCLC]; Ross, *Curr.Opin.Oncology*, 10:153-161 (1998) [melanoma]; Kreth, et al., *Cancer*, 79:370-379 (1997) 79:370-379 [gliomas]; and, Shaw, et al., *J.Neurooncol.*, 3 1:273-278 (1997) [gliomas].

Further, with knowledge provided by the invention of the most common genesis points for 9p21 derangements (exon 8 of the MTAP gene [Fig. 8; SEQ.ID.No.: 26]; the junction of exons 4 and 5 of the MTAP gene [*id.*], as well as a point between STS 3.21 and MTAP exon 8 Figs. 7 and 8; SEQ.ID.Nos. 25 and 26]), early detection and correlation to grade of advancement of cancers other than those specifically studied herein can be performed by those of ordinary skill in the art, especially through utilization of the techniques described herein for obtaining histological-grade specimens of tumors at early stages of development and application

of the highly sensitive PCR-ELISA technique for analysis of such specimens which is described herein.

Examples illustrating practice of the invention and providing data in proof of the principles underlying the invention are provided below. Neither these examples nor the disclosure above should be regarded as limiting the scope of the invention, which is defined by the claims appended hereto.

In the Examples, standard abbreviations (e.g., "ml" for milliliters; "n" for sample size and the like) are used unless otherwise noted.

EXAMPLE I

METHOD FOR OBTAINING HISTOLOGICAL-GRADE SPECIMENS OF TUMORS AT EARLY STAGES OF DEVELOPMENT

Tumor Samples. Ninety-five malignant brain tumors excised from human patients and provided to the glioma marker network were obtained from the Mayo clinic, Rochester, Minnesota (n=19), the Johns Hopkins University, Baltimore, Maryland (n=51), and the University of California at San Diego (n=25). Tumors were either frozen at -70°C directly after excision without further fixation (n=7 1), or were fixed in 10% neutral buffered formalin for 24-48 hours, then were immediately embedded in histologic grade paraffin according to standard procedures (n=24). Paraffin blocks were stored at room temperature until assayed. Representative slides were available from all paraffin-embedded tissue blocks and were examined microscopically to determine the tumor-rich parts of each specimen.

In addition, 16 xenograft tumors (established at Duke University) were investigated. The xenografts were derived from both childhood (n=5) and adult (n=11) primary malignant gliomas, and were propagated in nude mice to a diameter of approximately 2 cm. Excised tumors were stored at -70°C until analyzed.

NSCLC tumors were obtained from 50 randomly selected male human patients having a median age of 65 years. The tumors were paraffin-embedded as described above.

DNA Extraction. DNA from unfixed samples as well as from xenograft

tumors was extracted using a Qiagen Tissue Kit (Qiagen, Chatsworth, CA) according to the manufacturer's protocol. Between 25-75 mg of tissue were used in each extraction procedure. DNA was measured using a GeneQuant spectrophotometer (Pharmacia, Freiburg, Germany). DNA samples were stored at a concentration of 10 ng/µl at -20°C until used for PCR.

DNA from fixed tissues was extracted either from the paraffin-block or from 5 µm sections as follows: The top 100 µm of each paraffin block was shaved and discarded to avoid potential sample contamination with extraneous DNA adhering to the block surface during storage. Microscopically identifiable regions of tumor were scratched off the block to a depth of about 5-10 µm with a sterile scalpel blade or needle and placed in a sterile microcentrifuge tube. Alternatively, tumor-rich regions were scratched off the slide after identification in the representative H&E stained slide. Specimens were deparaffinized by incubation twice for 10 minutes in octane. To remove any remaining octane, the tissue pellets were washed twice with 100% ethanol and dried. Further DNA isolation was performed using a Qiagen Tissue Kit with the following modifications: the dried tissue pellet was resuspended in 320 µl ATL lysis buffer and 40 µl proteinase K (Sigma) stock solution (20 mg/ml) was added. Samples were then incubated at 37°C for 96 h with further addition of 40 µl proteinase K at 48 h. After digesting contaminating RNA with 2 mg/ml RNase A (Sigma, St. Louis, MO), DNA was then isolated by elution with 200 µl 10 mM Tris-HCL, pH 9.0.

EXAMPLE II

PCR-ELISA ANALYSIS OF TUMOR SAMPLES

Polymerase Chain Reaction (PCR). PCR-ELISAs were performed generally as described in Barker, *et al.*, *J.Neuro-Oncol.*, 31:17-23 (1997) and in Perry, *et al.*, *J.Neuropath.Exp.Neurol.*, 56: 999-1008 (1997), the disclosures of which are incorporated herein by reference. A total of 50 ng of DNA isolated from each sample was used for amplification.

The recently cloned pseudogene of MTAP (ΨMTAP) localized to chromosome 3q28, served as a control gene. Primers for p16INK4B exon 1, p16 exon

1α and exon 3, MTAP exon 8, and ΨMTAP, and for the sequence tagged sites 5B5 54F, 2F, 3.21, are listed in Table 1. Primers were selected to amplify exon 1 of p16 and exon 8 of MTAP. Primer sequences used to amplify MTAP exons 1-7 have been previously published. All primer pairs were designed to amplify PCR products of similar length between 197-250 bp, and all sense primers were biotinylated at the 5′ end (Integrated DNA Technology, Coralville, IA).

TABLE 1. *Primer Sequences Used for PCR and RT-PCR*

Marker	Sense Primer	Anti-sense Primer	Product Length
ΨMTAP	5'-AGGGACCTC GTTTTATCTC TTGA-3' (SEQ.ID.No.: 1)	5'-CTAGCATT TTCTTTCGGGGTCTG-3' (SEQ.ID.No.: 2)	216bp
MTAP exon 8	5'-AGTTTTC TGTTTTATTACCAA G-3' (SEQ.ID.No.: 3)	5'- GTCATTTGCTTTTCTTCTGTAT T-3' (SEQ.ID.No.: 4)	240bp

Marker	Sense Primer	Anti-sense Primer	Product Length
P15 exon 1	5'-GGAATTCTAGGCTGCGGAATGCGCGAGGAG-3' (SEQ.ID.No.: 5)	5'-ATCATGACCTGGATCGCGCGGCCTCCCGAAA-3' (SEQ.ID.No.: 6)	179bp
STS 5BS*	5'-TTCTTAGAATAATGGTAT-3' (SEQ.ID.No.: 7)	5'-TAAGGATATTTACATAG-3' (SEQ.ID.No.: 8)	181bp
P16 exon 1α**	5'-TCGGCGGCTGCGGAGAGGGGGAGAG-3' (SEQ.ID.No.: 9)	5'-TCCTCCAGAGTCGCCCGCCATCC-3 (SEQ.ID.No.: 10)	250bp
P16 exon 3	5'-CGATTGAAAGAACCAGAGAGG-3' (SEQ.ID.No.: 11)	5'-ATGGACATTTACGGTAGTGGG-3' (SEQ.ID.No.: 12)	196bp
STS 54F	5'-AAAGGAGTTGGATTGTG-3' (SEQ.ID.No.: 13)	5'-TTCTCACTCCCATTTTCATC-3' (SEQ.ID.No.: 14)	184bp
STS 2F	5'-TGAGAACTAGAGCTTGGAAG-3' (SEQ.ID.No.: 15)	5'-AACCCTCCTTCAAATCTGTA-3' (SEQ.ID.No.: 16)	248bp
STS 3.21	5'-AGGATGTTGAAGGGACATTG-3' (SEQ.ID.No.: 17)	5'TGTGTTGTGGACCTCTGTGC-3' (SEQ.ID.No.: 18)	200bp
GAPDH	5'-AAGAAGATGCGGCTGACTGTCGAGCCACAT-3' (SEQ.ID.No.: 19)	5'-TCTCATGGTTCACACCCATGACGAACATG-3' (SEQ.ID.No.: 20)	510bp
MTAP exons 1-4	5'-ATGGCCTCTGGCACCACCAC-3' (SEQ.ID.No.: 21)	5'-CTGTCAATGAACTGATCAATAATGAC-3' (SEQ.ID.No.: 22)	347bp

Marker	Sense Primer	Anti-sense Primer	Product Length
MTAP exons 5-8	5'-GACCACTATGAGACCTCAGTCCTTCTATGATG-3' (SEQ.ID.No.: 23)	5'-TTAATGTCTTGGTAATAAAACAGAAAACTGGG-3' (SEQ.ID.No.: 24)	505bp

*Annealing temperature: 45 °C **Annealing temperature: 64°C

A standard curve was established for each amplified sequence using DNA isolated from the normal lung fibroblast cell line WI-38 (for unfixed samples), or from formalin-fixed, paraffin-embedded normal placenta (for formalin-fixed, paraffin-embedded tumor samples). Zero to 100 ng of control DNA were amplified in each experiment to show the linearity of the PCR reaction and to provide a standard for comparison of amplified tumor sample DNA to normal DNA products. PCR was performed in a total of 50 µl containing normal or sample DNA, 2.0 mM MgCl2, 2.0 µl digoxigenin PCR Mix (200 µM each of dATP, dCTP, and dGTP, 190 µM dTTP and 10 µM digoxigenin-dUTP) (Boehringer Mannheim, Indianapolis, IN), 7.5-15 pmol of each primer, and SU AmpliTaq Gold? DNA Polymerase (Perkin Elmer, Branchburg, NJ) in the reaction buffer provided by the supplier.

Due to a very high GC content of p16 exon laα, amplification was performed in a reaction mixture containing 5% dimethylsulfoxide (DMSO). Samples were amplified through 27 cycles (fresh tissue) or 29-33 cycles (paraffin-embedded tissues) in a Perkin Elmer thermal cycler using the following parameters: initial denaturation at 94°C for 5 min, then 27 (29-33, respectively) cycles of 94°C denaturation for 1 min, annealing at 59°C for 1 min (64° in case of p16 exon lα), polymerization at 72°C for 1 min and a final extension step at 72°C for 7 min.

Ten µl of the PCR reactions were analyzed by electrophoresis at 100 V for 45 min in a 2% agarose gel in Tris-borate buffer containing 2 mM EDTA and 0.4 µg/ml ethidium bromide. Gels were photographed and in some experiments scanned. The intensities of the bands were analyzed using standard software.

Enzyme-Linked Immunosorbent Assay (ELISA). All samples were analyzed for p16 and MTAP deletions in a subsequent ELISA using primers for p16 exon 1 and exon 3, and MTAP exon 8. Polyvinyl microwell plates (Becton-Dickinson, Oxnard, CA) were pretreated with glutaraldehyde 0.1% at room temperature for 20 min, washed with PBS, and then coated with streptavidin 0.1 mg/ml (Sigma, St. Louis, MO) for 2 h at 37°C. Wells were washed with PBS, then filled with a 0.2 mg/ml sodium borohydrate solution for 10 min at room temperature, followed by a PBS wash. Wells were then treated with Triton-X100 0.1% for 30 min at room temperature, and then blocked with 1% bovine serum albumin (Sigma) and stored at 4°C until used.

The PCR products obtained were separated from free primers and unincorporated digoxigenin-dUTP using a Qiagen PCR purification kit according to the manufacturer's instructions. PCR products were eluted in a total volume of 100 µl 10 mM Tris-HCl, pH 9.0. Purified PCR samples were diluted 1:100 (1:25 in some cases when no ΨMTAP was visible on the gel) in BW buffer (6x SSC, 0.1% Tween-20), and 100 µl aliquots were added in triplicate to the streptavidin-coated plates, previously washed with PBS and BW buffer. After 1 h incubation at room temperature, wells were washed once with BW buffer and buffer B (800 mM NaCl, 100 mM Tris-HCl, pH 7.5, 0.5 % Genius? blocking reagent (Boehringer Mannheim), 5 mM maleic acid). Anti-digoxigenin antibody conjugated with horse radish peroxidase (150 U/ml, Boehringer-Mannheim) was diluted 1:1000 in buffer B and 100 µl aliquots were added to the wells and incubated for 30 min at 37°C. Plates were then washed once with buffer B and twice with buffer A (100 mM Tris-HCl, pH 7.5, 800 mM NaCl). To develop the ELISA, 100 µl of substrate solution (equal parts TMB and H202) were added to each well, followed by a 5 min incubation at room temperature. The reaction was stopped by addition of 100 µl of iM o-phosphoric acid. Plates were read in a microtiter plate spectrophotometer at a wavelength of 450 nm.

Gene Dosage Quantification. A regression line for each target sequence was calculated according to the standard curve data obtained in the ELISA from normal placenta DNA. The quotient of MTAP or p16 PCR product absorbance values (OD)

divided by that of the ΨMTAP reference gene, derived from the standard curve for control DNA, was used to normalize the values. The tumor sample gene dosage ratio was calculated from the equation (example):

$$\text{Sample MTAP gene dosage ratio} = \frac{\text{sample OD MTAP/sample OD } \Psi\text{MTAP}}{\text{control OD MTAP/control OD } \Psi\text{MTAP}}$$

PCR for each sample was done at least twice. Inter-assay coefficient of variation of the OD was <15%. A standard curve and calculation of the normalization factor from control DNA was done with each PCR assay. Only sample OD values in the linear range of the standard curves were considered for calculation. A sample was considered to have homozygous MTAP or p16 deletion when the normalized gene dosage ratio was <0.3. This criterion was chosen prior to the initiation of sample analysis based on previous reports utilizing differential PCR to detect p16 homozygous deletions.

Frequency of Homozygous Deletions of p16 and MTAP. Homozygous p16 deletions occurred in 25 out of 95 samples (26%), while homozygous MTAP deletions were observed in 22 out of 95 cases (23%) (Table 2). Fourteen samples (15%) had a co-deletion of both genes, 11 (12%) had only a p16 deletion, and 8 (8%) had only MTAP deletions (Fig. 1, Table 2).

TABLE 2. *Incidence of Homozygous MTAP/p16 Deletions in Primary Brain Tumor Samples*

		GENE MARKER			
		MTAP+	MTAP+/ P16-	MTAP-/ P16+	MTAP- /P16-
TOTAL # PATIENTS:	95	62/95 (65%)	11/95 (12%)	8/95 (8%)	14/95 (15%)
HISTOLOGICAL SUBTYPES:	TOTAL	%	%	%	%
Low-grade Astrocytoma	9	7/9 (78%)	0/9 (0%)	2/9 (22%)	0/9 (0%)
Anaplastic Astrocytoma	5	3/5 (60%)	0/5 (0%)	1/5 (20%)	1/5 (20%)
Oligodendro-gliomas	14	12/14 (86%)	0/14 (0%)	1/14 (7%)	1/14 (7%)
Oligoastro-cytomas	7	4/7 (57%)	0/7 (0%)	3/7 (43%)	0/7 (0%)
Glioblastomas	60	36/60 (60%)	11/60 (18%)	1/60 (2%)	12/60 (20%)

Relation to Grade. Classification of the tumor samples, with regard to histological type and grade, showed that homozygous p16 deletions were found predominantly in grade IV glioblastomas (23 out of 60, 38%; $p<0.0001$). Furthermore, the frequency of p16 deletions was higher in astrocytic tumors (32%) compared to oligodendrogliocytic tumors (7%), and was not found in mixed, oligo-astrocytomas (Table 2). Homozygous MTAP deletions could be detected in all histological types, but co-deletions with p16 were confined to high-grade glioblastomas (55% of all MTAP deletions). Homozygous MTAP deletions without p16 deletions were characteristics of lower grade brain tumors ($p=0.0005$), with no obvious predominance of a distinct histological subgroup. Only one glioblastoma showed an MTAP-/p16+ configuration, and in this sample the values for the MTAP/ΨMTAP ratio (0.28) and the p16/ΨMTAP ratio (0.43) were very close to the 0.3 cut-off value. These results indicate that a homozygous MTAP deletion without a p16 deletion is

associated with low-grade malignant gliomas.

Sixteen brain tumor xenografts were studied with the same primer sets (Fig. 3). Breakpoints between MTAP exons 4/5 and 7/8 were also found in the xenografts, as was the breakpoint between p15 and p16. None of the childhood tumors showed a MTAP deletion, whereas most of the adult tumors showed a partial or complete deletion of the MTAP gene. Furthermore, p16 was deleted in all but one of the adult tumors, but only in a single childhood tumor (Fig. 3). An inverse relation between p15 and p16 could be observed in these childhood gliomas. These results suggest that the isolated deletion of p15 is characteristic of childhood gliomas, whereas p16 and MTAP deletions distinguish adult gliomas.

EXAMPLE III

FINE MAPPING OF 9p21 DERANGEMENTS IN BRAIN TUMORS

Fine Mapping. Fine mapping of the region between p15 and MTAP was performed in 37 primary tumors and in 16 xenografts using primers for recently identified sequence tagged sites (STS) as well as for the distinct exons of p15, p16, and MTAP (Table 1). The PCR was performed as described above; the gels were scanned and analyzed. Results were normalized according to the standard curves and gene dosage ratios compared to the ΨMTAP values were calculated. A homozygous deletion was postulated, when the normalized gene dosage ratio was <0.3. In some borderline cases, an additional ELISA was performed.

To investigate the locations of the different deletions, we performed fine mapping of the region between p15 and MTAP in 36 brain tumor samples. Recurrent breakpoints within the MTAP gene were detected between exons 4/5 (2 cases) and between exons 7/8 (4 cases). Histologic grade I-III tumors had much smaller deletions than grade IV (Fig. 2). The minimally deleted region encompassed the 3'-region of MTAP, with the breakpoints mentioned above. Homozygous deletion of MTAP in high-grade glioblastomas included the p16 gene in 10 out of 11 cases (Fig. 2). Only in one sample (#799) did the deletion start centromeric of p16, and involved the p15 gene. Two tumor samples showed a centromeric breakpoint between p15 and

p16, and a telomeric breakpoint beyond MTAP (#802 and #803). In addition, breakpoints between MTAP exons 4/5 (#T303, #723) and between STS 3.21 and MTAP exon 8 (#756, #801) could be identified in samples from patients with glioblastomas (Fig. 2). This suggests the existence of fragile sites between STS 3.21 and MTAP exon 8, between MTAP exons 7 and 8, and between MTAP exons 4 and 5.

EXAMPLE V

MEASUREMENT OF PROTEIN EXPRESSION FROM DERANGED 9p21 GENES

Western Blot Analysis. Xenograft tumors were dissected using a razor blade. The sections were crushed in microfuge tubes containing 500 µl of RIPA-M buffer (50 mM NaCl, 50 mM Tris pH 7.4, 0.5% NP-40, 1 mM EGTA, 1mM Na3VO4, 1mM NaF, 1 mg/ml aprotinin, 1 mg/ml leupeptin, 1mM PMSF). Samples were further disrupted by passing several times through an 18G needle, then a 22G needle, a 27G, and incubated at 4°C overnight. Then, samples were spun down at 12000g for 10 min., and the protein in the supernatant was quantified with a Pierce Coomassie Plus? Protein Assay Reagent (Rockford, IL). After boiling in sample buffer for 5 min., equal amounts of 50 µg were loaded onto 14% Tris-glycine gels (Novex, San Diego, CA), separated at 125V for 90 min, and transferred to PVDF-membranes (Millipore, Bedford, MA). Membranes were blocked overnight with 1% casein-blocking solution, and probed for 1 h at 20°C with either a monoclonal mouse-anti-human p16-antibody (Pharmingen, San Diego, CA), or a polyclonal chicken antibody generated against recombinant human MTAP. Bound antibody was detected using Western-Light chemiluminescence detection kit (Tropix, Bedford, MA).

Reverse Transcriptase-PCR (RT-PCR). Total RNA was extracted from tumor tissues with a Qiagen? kit. About 25 mg of tissue were homogenized by aspirating several times with a 1cc syringe through a 22G needle in lysis buffer. RNA was eluted from spin columns in 30 µl DEPC-treated water. One µg of RNA was transcribed into single-stranded cDNA using a first-strand cDNA synthesis kit (Gibco-BRL, Gaithersburg, MD), and random primers.

RT-PCR was carried out in a total volume of 25 µl including 3 µl cDNA 2.0

mM MgCl2, 200 µM dNTPs (Sigma, St. Louis, MO), 7.5 pmol of each primer, and 5 U AmpliTaq Gold? DNA Polymerase (Perkin Elmer, Branchburg, NJ) in the reaction buffer provided by the supplier. PCR conditions were as follows: Initial 5 min 94°C, 35 cycles of 94°, 30 sec., 58°C, 30 sec., 72°, 1 min., and a final extension of 7 min. at 72°C. Primers for GAPDH served as positive control (Table 1). PCR products were electrophoresed on a 2% agarose gel as indicated above. Chi-square test was used to determine statistical significance.

p16 and MTAP Protein Expression in Xenograft Tumors. Two out of 4 samples with an intact p16 gene did not express the p16 protein (Fig. 4a). This result is in accordance with previous findings that p16 is highly regulated in most human tumors and that homozygous deletion is not the only mechanism of inactivation. On the other hand, MTAP protein was found in all samples with an intact gene (Fig. 4b). Furthermore, two tumors (#245 and #270) with deletions of MTAP exons 5-8 and MTAP exon 8, respectively, produced immunoreactive MTAP proteins. The 32 kDa immunoreactive band in the tumor with an isolated exon 8 deletion was indistinguishable from the wild type MTAP protein.

EXAMPLE VI

mRNA MTAP EXPRESSION ANALYSIS TO CONFIRM EFFECT OF DELETION

MTAP mRNA Expression in Xenografts. Because the antisense primer for the amplification of MTAP exon 8 is 225 bp downstream of the coding sequence, it was possible that the deletion in sample #270 is out of the coding sequence in the 3'-untranslated region. This could result in the synthesis of a normal molecular weight MTAP protein. We therefore performed RT-PCR with two different primer pairs. MTAP1x4 includes exactly the coding sequence from MTAP exon 1 to MTAP exon 4 (bases 122-468 according to the GeneBank mRNA sequence U22233), whereas MTAP5x8 amplifies exactly the region from the beginning of MTAP exon 5 to the end of the coding sequence in MTAP exon 8 (bases 469-973). We could amplify PCR-products with primers MTAPlx4 in both sample #245 and sample #270, but amplification with MTAP5x8 primers did not result in a PCR-product in either tumor

(Fig. 5). Sample #696 served as a positive control. These results confirm the MTAP deletion and western blotting data, and suggest the existence of truncated forms of MTAP in some gliomas.

EXAMPLE VII

ANALYSIS OF NSCLC TUMOR SAMPLES

Tumor samples from 50 randomly selected male human patients were obtained, then prepared for analysis and analyzed as described in preceding Examples.

Guided by microscopic examination of representative slides, the DNA samples extracted from NSCLC specimens contained (30% contaminating normal tissue DNA. Homozygous MTAP deletions were detected in 19 of the 50 samples (38%) from patients with NSCLC (Table 3). Adenocarcinomas showed a higher frequency of homozygous deletions (11 of *25,* 44%) than squamous cell carcinomas (6 of 21, 29%). Two of 4 large cell carcinoma samples were MTAP-deleted. No correlation between homozygous deletions and the age of the patients was evident.

In contrast to the results for MTAP, homozygous deletions of p16 exon 1a__ occurred in only 9 of 50 NSCLC samples (18%), including 4 of 25 adenocarcinomas (16%), 4 of 21 squamous cell carcinomas (19%) and 1 of 4 large cell carcinomas (Table 3). No patient had a p16 exon 1aα deletion without a deletion of MTAP cxon 8, but 10 samples had homozygous MTAP deletions without deletions of pl6 exon 1 (the exon closest to MTAP).

TABLE 3 *Incidence of Homzygous MTAP/p16 Deletions in NSCLC*

MTAP Deletions:	
Total	19/50 (38%)
Adenocarcinoma	11/25 (44%)
Squamous cell carcinoma	6/21 (29%)
Large cell carcinoma	2/4 (25%)

p16 Deletions (exons 1aα, 3):	
Total	9/50 (50%)
Adenocarcinoma	4/25 (16%)
Squamous cell carcinoma	4/21(19%)
Large cell carcinoma	1/4 (25%)

Several samples had a p16/ΨMTAP ratio of> 1.25. These tumors may have

had increased ploidy of chomosome 9, with or without a breakpoint between p16 and MTAP. On chromosome 9p2l, p16 and MTAP lie in tail-to-tail orientation at a distance of approximately 100 kb. Because exon 3 of p16 lies closer (i.e. more telomeric) than exon 1 to MTAP exon 8, p16 exon 3 was amplified with specific primers in the 10 samples with deleted MTAP but intact p16 exon 1. None of these samples had a deletion of the p16 exon 3 region that encompasses the coding sequence.

To identify the smallest deleted region in the NSCLC samples with p16+/MTAP- status, a fine mapping analysis by quantitative PCR was performed according to the protocol described above. Consistent with findings in brain tumors described in the preceding examples, a frequent telomeric breakpoint was found within the MTAP gene between MTAP exon 4 and 5.

The invention having been fully described, modifications and extensions thereof may become obvious to those of ordinary skill in the art. All such modifications and extensions are within the scope of the invention.

SUMMARY OF SEQUENCES

SEQ.ID.No.: 1 is a primer utilizable in the method of the invention.

SEQ.ID.No.: 2 is a primer utilizable in the method of the invention.

SEQ.ID.No.: 3 is a primer utilizable in the method of the invention.

SEQ.ID.No.: 4 is a primer utilizable in the method of the invention.

SEQ.ID.No.: 5 is a primer utilizable in the method of the invention.

SEQ.ID.No.: 6 is a primer utilizable in the method of the invention.

SEQ.ID.No.: 7 is a primer utilizable in the method of the invention.

SEQ.ID.No.: 8 is a primer utilizable in the method of the invention.

SEQ.ID.No.: 9 is a primer utilizable in the method of the invention.

SEQ.ID.No.: 10 is a primer utilizable in the method of the invention.

SEQ.ID.No.: 11 is a primer utilizable in the method of the invention.

SEQ.ID.No.: 12 is a primer utilizable in the method of the invention.

SEQ.ID.No.: 13 is a primer utilizable in the method of the invention.

SEQ.ID.No.: 14 is a primer utilizable in the method of the invention.

SEQ.ID.No.: 15 is a primer utilizable in the method of the invention.

SEQ.ID.No.: 16 is a primer utilizable in the method of the invention.

SEQ.ID.No.: 17 is a primer utilizable in the method of the invention.

SEQ.ID.No.: 18 is a primer utilizable in the method of the invention.

SEQ.ID.No.: 19 is a primer utilizable in the method of the invention.

SEQ.ID.No.: 20 is a primer utilizable in the method of the invention.

SEQ.ID.No.: 21 is a primer utilizable in the method of the invention.

SEQ.ID.No.: 22 is a primer utilizable in the method of the invention.

SEQ.ID.No.: 23 is a primer utilizable in the method of the invention.

SEQ.ID.No.: 24 is a primer utilizable in the method of the invention.

SEQ.ID.No.: 25 is the sequence of a cosmid including sequences spanning 9p21 (ATCC AC00047).

SEQ.ID.No.: 26 is a genomic sequence for the gene encoding methylthioadenosine phosphorylase.

The invention claimed is:

CLAIMS

1. A method for diagnosis of, and determining a prognosis for, cancer causatively associated with derangements of chromosome 9p21, the method comprising:

(a) determining whether any portion of the 9p21 chromosome including and telomeric to STS 3.21 is deleted; and,

(b) determining whether any portion of the 9p21 chromosome centromeric to STS 3.21 is deleted;

wherein a positive finding in step (a) and a negative finding in step (b) are indicative of a cancer at an early stage of tumor development; and,

wherein further a positive finding in step (b) is indicative of a cancer at an advanced stage of tumor development.

2. A method for diagnosis of, and determining a prognosis for, cancer causatively associated with derangements of chromosome 9p21, the method comprising:

(a) determining whether any portion of the gene encoding MTAP is deleted; and,

(b) determining whether any portion of the 9p21 chromosome centromeric to STS 3.21 is deleted;

wherein a positive finding in step (a) and a negative finding in step (b) are indicative of a cancer at an early stage of tumor development; and,

wherein further a positive finding in step (b) is indicative of a cancer at an advanced stage of tumor development.

3. The method according to Claim 2 wherein step (a) comprises determining whether exon 8 of the gene coding for MTAP is deleted.

4. The method according to Claim 2 wherein step (a) comprises determining whether the region from exon 4 to exon 5 of the gene coding for MTAP is deleted.

5. The method according to Claim 2 wherein step (b) comprises determining whether any portion of the gene coding for p16 is deleted.

6. A method for diagnosis of, and determining a prognosis for, cancer causatively associated with derangements of chromosome 9p21, the method comprising:

(a) determining whether any portion of the gene encoding MTAP is deleted; and,

(b) determining whether any portion of the gene coding for p16 is deleted;

wherein a positive finding in step (a) and a negative finding in step (b) are indicative of a cancer at an early stage of tumor development; and,

wherein further a positive finding in step (a) and a positive finding in step (b) is highly indicative of a cancer at an advanced stage of tumor development.

7. The method according to any of Claims 1, 2 and 6, further comprising the use in step (a) of primer pairs selected from the group of nucleotides consisting of SEQ.ID.No.: 3 and 4; SEQ.ID.No.: 21 and 22, and SEQ.ID.No.: 23 and 24.

8. The method according to any of Claims 1, 2 and 6, further comprising the use in step (b) of primer pairs selected from the group of nucleotides consisting of SEQ.ID.No.: 5 and 6; SEQ.ID.No.: 7 and 8; SEQ.ID.No.: 9 and 10; SEQ.ID.No.: 11 and 12; SEQ.ID.No.: 13 and 14; SEQ.ID.No.: 15 and 16; and, SEQ.ID.No.: 17 and 18.

9. The method according to any of Claims 1, 2 and 6, further comprising the use of the primer pair of SEQ.ID.No.: 1 and 2 as a control for step (a).

10. The method according to any of Claims 1, 2 and 6, wherein the cancer is a glioma.

11. The method according to any of Claims 1, 2 and 6, wherein the cancer is a primary lymphoid malignancy.

12. The method according to any of Claims 1, 2 and 6, wherein the cancer is non-small cell lung cancer.

13. The method according to any of Claims 1, 2 and 6, wherein the cancer is a melanoma.

14. The method according to any of Claims 1, 2 and 6, wherein the cancer is a head and neck cancer.

15. The method according to any of Claims 1, 2 and 6, wherein the cancer is ovarian cancer.

16. The method according to any of Claims 1, 2 and 6, wherein the cancer is bladder cancer.

17. The method according to any of Claims 1, 2 and 6, wherein the cancer is a chondrosarcoma.

18. A kit for use in the methods of any of Claims 1, 2 and 6, the kit comprising: primers for use in step (a) of the method; primers for use in step (b) of the method; and, at least one set of primers for use as a control, and a staging reference.

19. The kit according to Claim 18, wherein the primers for use in step (a) are

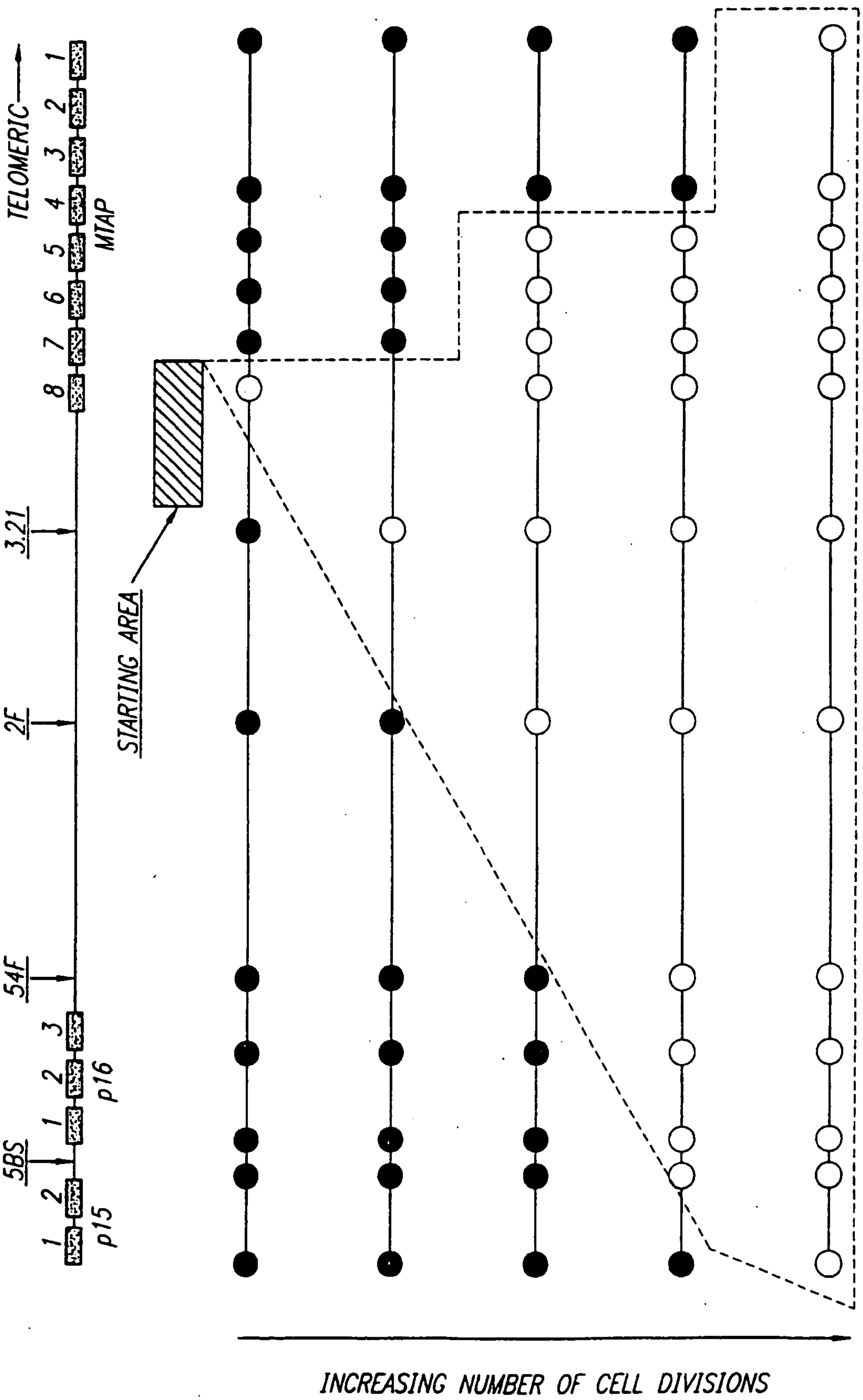
selected from the group of nucleotides consisting of SEQ.ID.No.: 3 and 4; SEQ.ID.No.: 21 and 22, and SEQ.ID.No.: 23 and 24.

20. The kit according to Claim 18, wherein the primers for use in step (b) are selected from the group of nucleotides consisting of SEQ.ID.No.: 5 and 6; SEQ.ID.No.: 7 and 8; SEQ.ID.No.: 9 and 10; SEQ.ID.No.: 11 and 12; SEQ.ID.No.: 13 and 14; SEQ.ID.No.: 15 and 16; and, SEQ.ID.No.: 17 and 18.

21. The kit according to Claim 18, wherein the primer for use as a control consist of SEQ.ID.No.: 1 and 2 as a control for step (a).

22. The kit according to Claim 18, wherein the staging reference comprises the data set forth in Figure 1.

FIG. 1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2
3
4
5
6
7
8
MTAP
3.21
2E
54F
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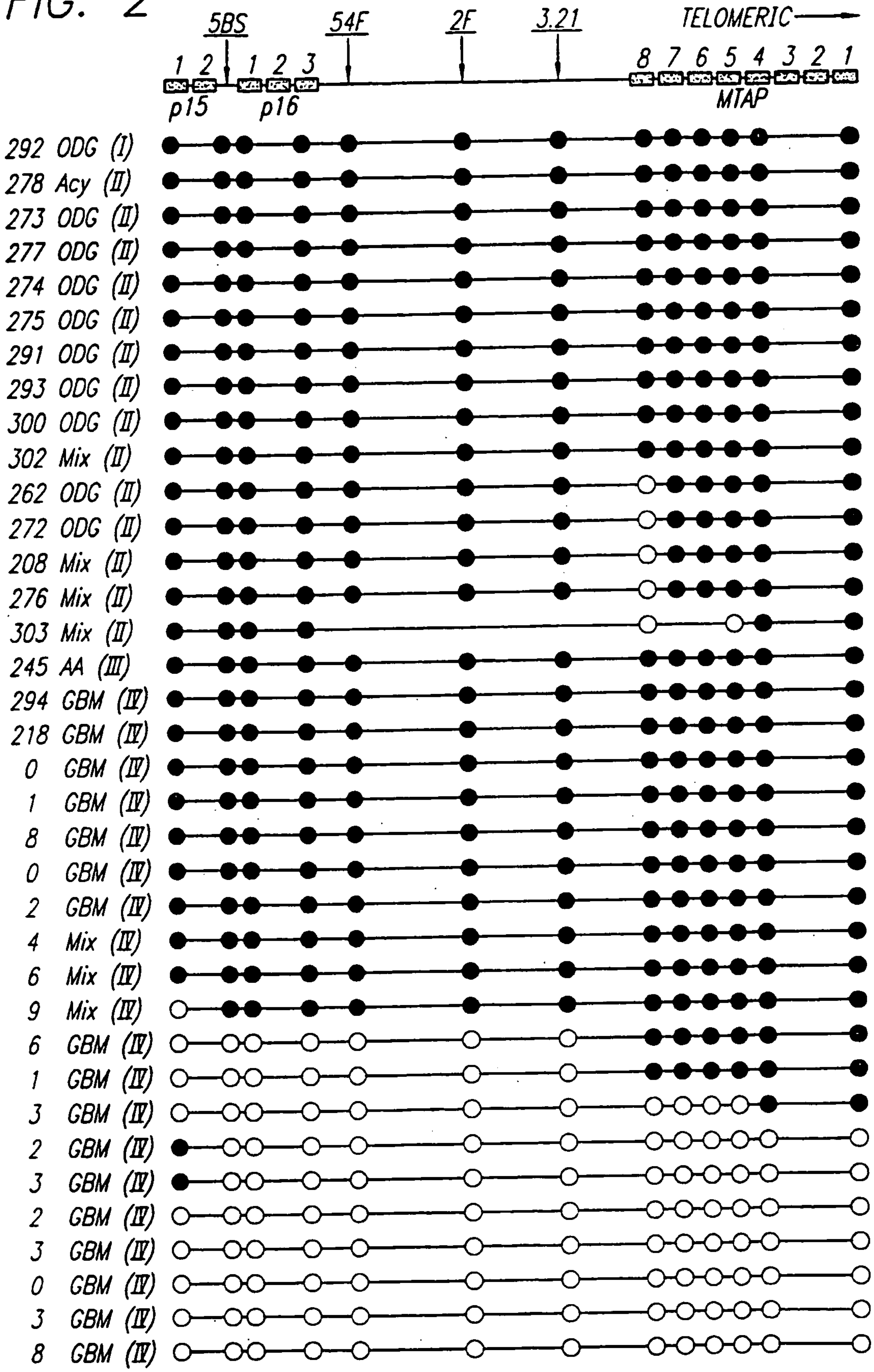
p16
5BS
2
1
p15
STARTING AREA
INCREASING NUMBER OF CELL DIVISIONS

2/19

FIG. 2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54F
2F
3.21
TELOMERIC
1 2
1 2 3
8 7 6 5 4 3 2 1
p15
p16
MTAP
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278 Acy (II)
273 ODG (II)
277 ODG (II)
274 ODG (II)
275 ODG (II)
291 ODG (II)
293 ODG (II)
300 ODG (II)
302 Mix (II)
262 ODG (II)
272 ODG (II)
208 Mix (II)
276 Mix (II)
303 Mix (II)
245 AA (III)
294 GBM (IV)
218 GBM (IV)
0 GBM (IV)
1 GBM (IV)
8 GBM (IV)
0 GBM (IV)
2 GBM (IV)
4 Mix (IV)
6 Mix (IV)
9 Mix (IV)
6 GBM (IV)
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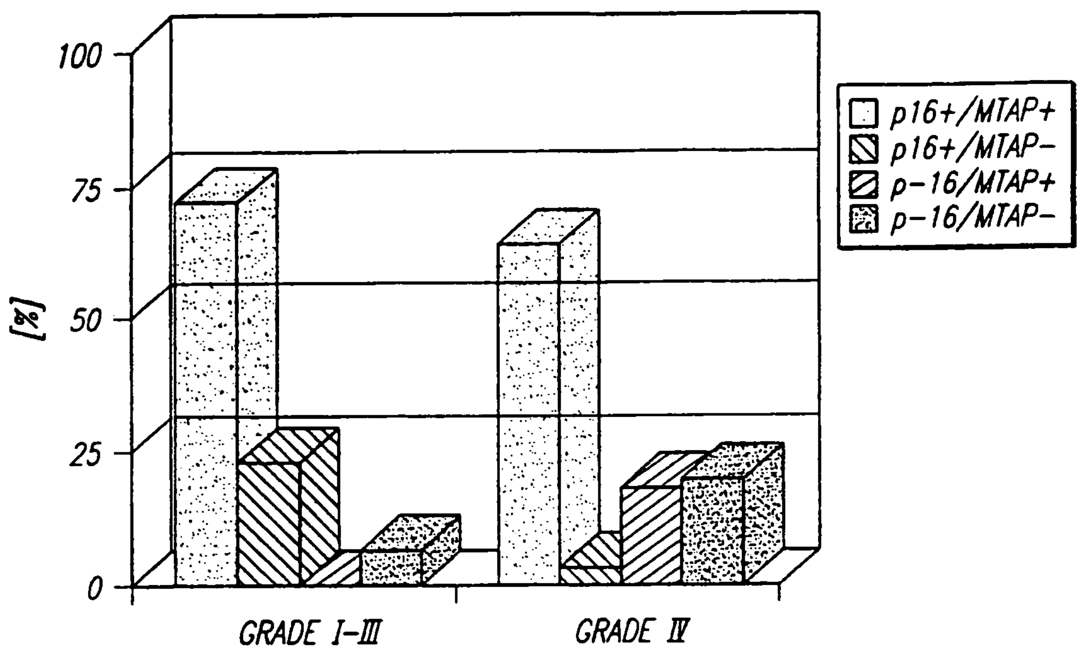
FIG. 3


100
75
50
25
0
[%]
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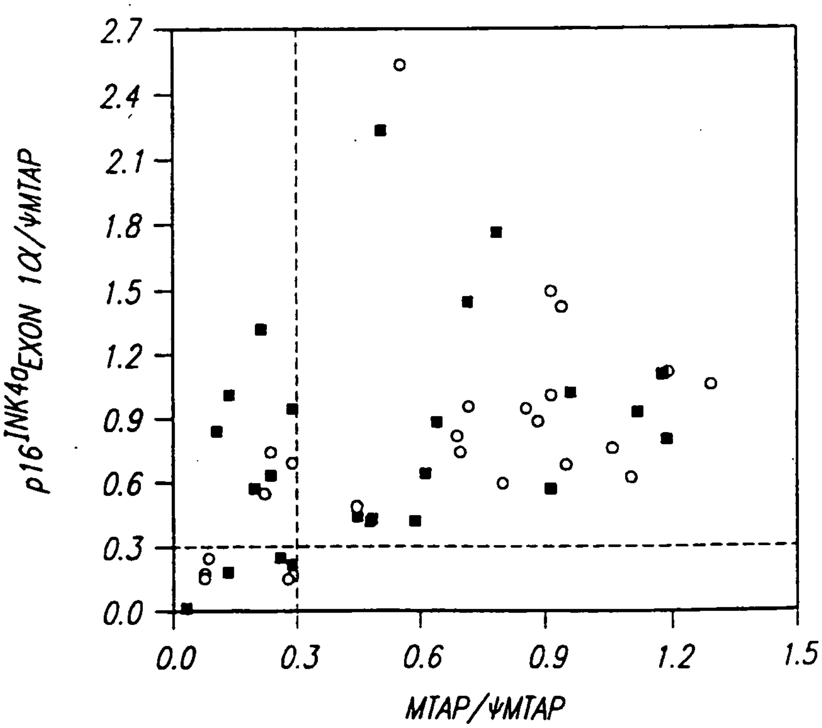
p−16/MTAP+
p−16/MTAP−
GRADE I–III
GRADE IV

FIG. 6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1.5
1.2
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0.6
0.3
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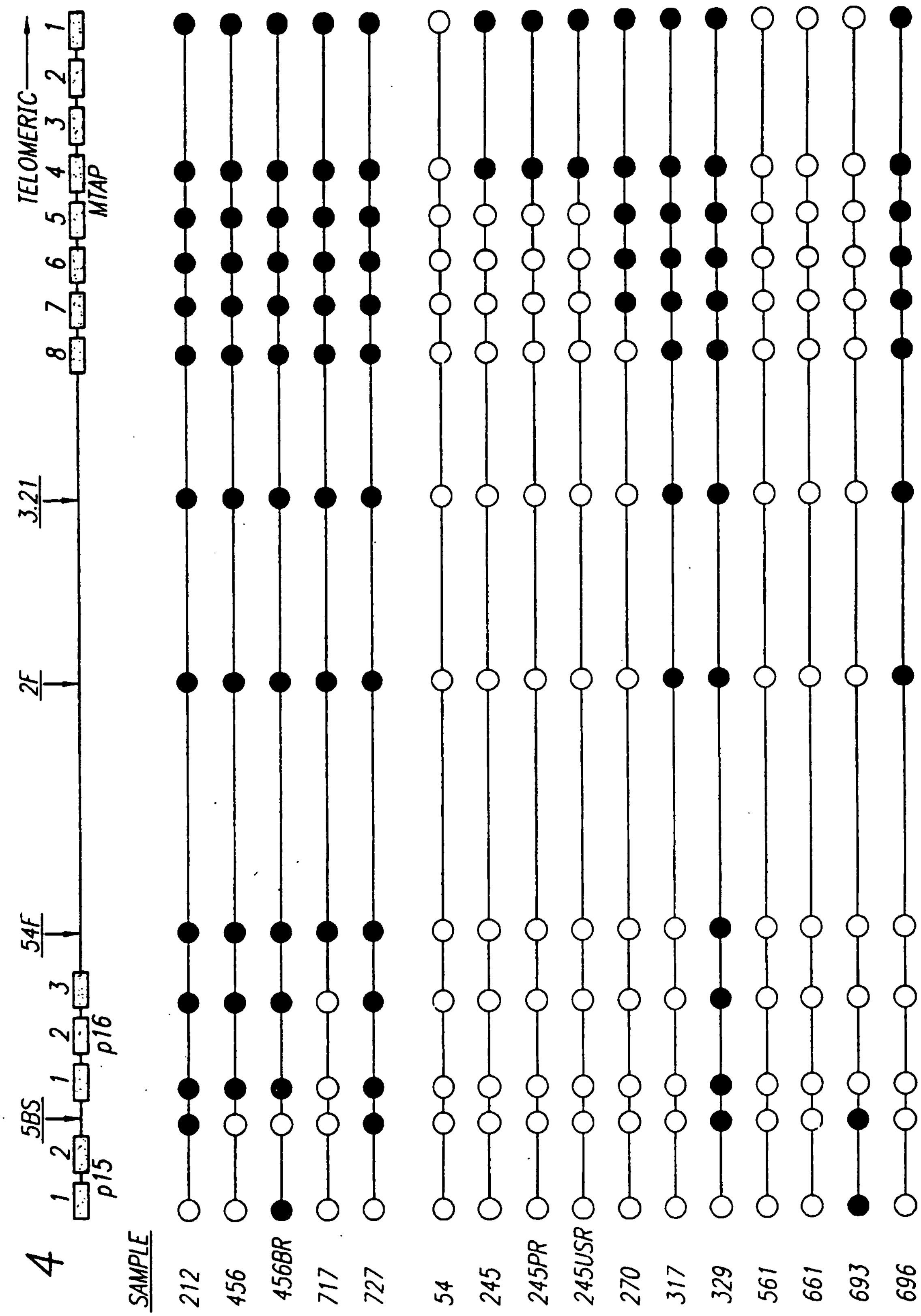
0.3
0.6
0.9
1.2
1.5
MTAP/ΨMTAP


TELOMERIC
MTAP
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3.21
2F
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3 2 1
p16
5BS
2 1
p15
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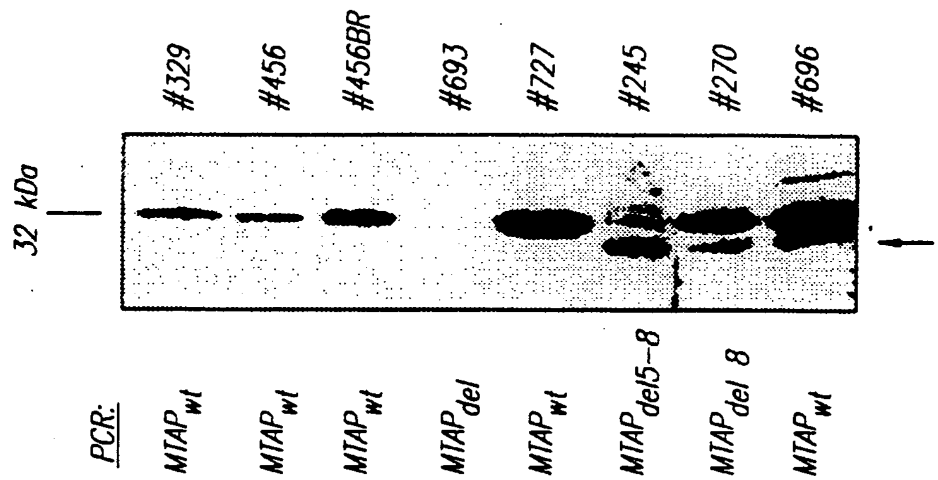
693
696

FIG. 4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#727
#245
#270
#696
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$MTAP_{wt}$
$MTAP_{wt}$
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$MTAP_{wt}$
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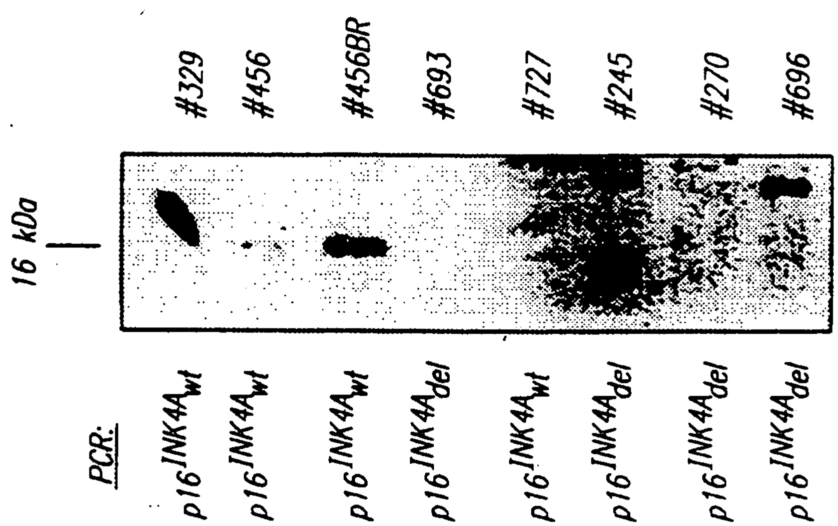
$MTAP_{del\ 8}$
$MTAP_{wt}$

FIG. 5b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#456
#456BR
#693
#727
#245
#270
#696
PCR:
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$p16^{INK4A}_{wt}$
$p16^{INK4A}_{wt}$
$p16^{INK4A}_{del}$
$p16^{INK4A}_{wt}$
$p16^{INK4A}_{del}$
$p16^{INK4A}_{del}$
$p16^{INK4A}_{del}$

FIG. 5a

FIG. 7-1

LOCUS AC00004741599 bpDNA HTG 27-MAY-1999
DEFINITION Homo sapiens chromosome 9 clone c110_c20 map 9p21, WORKING
DRAFT
SEQUENCE, 1 ordered pieces.
ACCESSION AC000047
NIDg4895268
VERSIONAC000047.4 GI:4895268
KEYWORDSHTG; HTGS_PHASE2.
SOURCE human.
ORGANISM Homo sapiens
Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Mammalia;
Eutheria; Primates; Catarrhini; Hominidae; Homo.
REFERENCE 1 (bases 1 to 41599)
AUTHORS Burian,D.M., Tilahun,Y., Mitchell,N. and Roe,B.A.
TITLECombined overlapping sequence of cosmids c110 and c20 from human
chromosome 9q21
JOURNAL Unpublished (1996)
REFERENCE 2 (bases 1 to 41599)
AUTHORS Sveen,L., Olopade,F.I. and Rowley,J.D.
JOURNAL Unpublished (1996)
REFERENCE 3 (bases 1 to 41599)
AUTHORS Roe,B.A.
TITLEDirect Submission
JOURNAL Submitted (30-OCT-1996) Department Of Chemistry And
Biochemistry,
The University Of Oklahoma, 620 Parrington Oval, Room 208, Norman,
OK 73019, USA
COMMENT[WARNING] On Jun 15, 1999 this sequence was replaced by a newer
version gi:5069485.
On May 27, 1999 this sequence version replaced gi:4887259.
* NOTE: This is a 'working draft' sequence. It currently
* consists of 1 contigs. Gaps between the contigs
* are represented as runs of N. The order of the pieces
* is believed to be correct as given, however the sizes
* of the gaps between them are based on estimates that have
* provided by the submittor.
* This sequence will be replaced
* by the finished sequence as soon as it is available and
* the accession number will be preserved
* 141599: contig of 41599 bp in length.
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BASE COUNT13477 a 8216 c 8242 g 11664 t
ORIGIN
1 tctttacagg gtggcaggac agagtgagtg caagcagggt acatgccaca tgcttataaa
61 accatcagat cttgtgagac tcactcattg tcatgagaac aacatgggga aaccaccccc
121 atccaattat ctccacctgg tcccgccctt gacacgtggg tattatgcag attacaattc
181 aaggtgagat ttggatggga acacagagcc aaaccatatc agtactggta caaaaacaga

FIG. 7-2

```
 241 aacacaaatc aatggaacag agtagagaac ccagaaataa agctgcacac ctacaatcat
 301 ctgatctttg acaaagttga caaaaacaag caatggggaa aggactccct attcaataaa
 361 tggtgctggg ataactggct agccatatac agaagactga aactggaccc cttccttata
 421 ccatatacaa aaatcaactc aggatgcatt aaacacttaa atgtaaaacc tagaactata
 481 aaaaccctag aagaaaacct aggaaatacc attctagaca taggccttgg caaatatatc
 541 atcaggaaga ctccaaaagc aattgcaaca agaacaaaaa ttgacaagtg ggacctaatt
 601 aaactaaaga tcttctgcac agcaaaagaa actataaaca gattaaacag acaacctaca
 661 gaatgggaga aaatatttgt aagctatgca actgactcta atatccagaa tctataagga
 721 acttaaacaa attaacaagg aaaaaaattt aaaaatgggc aaacgaaatg aacagacact
 781 tttccaaaga agatgtacat gcagccaaca agcatatgac aaaaatgctc aacatcacta
 841 ataattaaac aaatacaaat caaagccaca atgaaatacc atatcacacc agtcagaatg
 901 gctactatta aaaagccaaa aatatcagaa gctggtgaga ttgcagggaa aatgggacac
 961 taatacactg ctggtgggaa tgtaaattag ttcagccact gtggaaagcg gtttggagat
1021 ttctcaaagg atttaaaaac aaaactacca ttcaacccag caatcccatt actgggtata
1081 tacccaaacg aatataaact gttcaaccat aaagactcat gcatgcttat gttcatcaca
1141 acactattca caatagcaaa gacatggaat caaccttgat gcccatcaac agtggactgg
1201 gtaaagaaaa agaggtaaat atacatcacg caatactatg cagccatgag aaagaacaaa
1261 ataatgtctt ttgcagcagc atggatgtag ctggagacta ttatcctaag taaactaacg
1321 caagaacaga aaaccaaata cctcatattc tcacttataa gtagaagcta aacactgagt
1381 acacatgaac acaaagaagg gaacaactga tgccaaggcc cacctaagga tgcagagtga
1441 gaggagagtt aggattgaaa aactacctct taggtactat gcttatgacc tgggtgatgg
1501 aataatctgt ataccaaact ccatgacata cagtttacga ttgtaacaca cctgcacagg
1561 catccctaaa acaaaacttg taaagaaaat accaaaatac acattgacaa aagaaaaaga
1621 agatactaaa gacaacctaa ataaagagag gtcttcatag atggaaaggc tcaatgttac
1681 aaaaatgtta gttctcctta atataaacta tccaatacaa ttccagttta aaaaattgaa
1741 gagttgagcc ttttaaattt ttttagtatt cataacaaaa acaattttaa gtttaatagc
1801 acaggaggaa aagtaatgaa gcttgaaaat taaaagggat acagaatcct aatgacactt
1861 tgattgacta tctcataaag aatcagacat caggcacaga aaattgccac atttagatt
1921 cttggctact tgcattttgc tagtttttca tatgattatt aactataaaa cctataaacg
1981 agcttttatt aggaatcaaa gaaagggaat aaaggactag ggaggatacc tcaacctcct
2041 ttgccaggtg tatggagttt ttaaaaaagg ttagtagcag ctataagggc aaaggaaaac
2101 aactgagtct caggggtttg ccacaaggag gaaatgaggt tatgaggaac ccacatgggc
2161 ctggagaagc ttgtgacatt ttctcatagg ctatttacag ccaatgccat cacaatacaa
2221 ataaatttaa aacagtgaag aaaaacagaa gtaagctagt tttactgatt gtagagatta
2281 ctctgtgtgc tatgatcaag ttccaaattt gtttgagttt tctagctgct agaaataaca
2341 aaaaggaaaa tatgacaaat gttacagtgt tcaaaagtag aaatgaggca acatgttaag
2401 tccacatgaa atccctaagt agaatgtatg agaaatagct ttcagtctgg gttcctacag
2461 gcaaggaaca ggtgccatct ccagctgagt caagcaaaag agtaattcgc taaacgatat
2521 tgcaatagct cacaaaatct actaggaaga actgaagtag aggctcaagg ctaagccact
2581 aggaaagaca cactgaagca tgttttagaa ttggtctgga gataaaacag cttcctccag
2641 ttccacagat actaccattt ccacctgcta agctagtgtc ccttcaaacc cttgtttcct
2701 gtagctcatg agtcagatcc catctggtag catttagttc agagtcttaa gtcgcatgcc
2761 tatgccctac ctgcaaaaga ggaaggaaaa gtaagcatct ggtattttta gctcctataa
2821 tggatcatcc acacttagca catgtctgta atactatgaa ttccccaaac ataaaaacta
2881 gttatactgg tacacaacca aaacaacatg acaaatgtga caataaccat acaccatcca
2941 ctttcaagtt ttctacataa ccagtgaagt caatccttag agggacttaa attaagatta
3001 caaattgtta cttgttccct tatgaacttt ctgacctaag tattctcaat ccaataatgt
3061 agttataatc ctgcatttat ttactgtttt catcagggtt tttaatacat ccatatatgt
3121 aactttcttg gatcaattta acttggtcta ggttattctt ggatctacca gaacagaacc
3181 cagaatgcct ggaggtgtgg atagaataga aaatctcttt tattaccttt aagggctgtt
3241 aaacaaaact gaattcacca gttacaaaag caaatgatta aggtaagaac agaaatatca
3301 acacattatg cttgtttact ttaaaatgaa actatcttct atataaataa aaactgaaat
3361 tcccaccaaa atgtaaagaa tttaaaaggt aaattgtaaa cattataaaa aggcattgtg
```

FIG. 7-3

3421 gaaaagatga attcaactaa atgtgcagct atctgaaaat gaggctgtgt tgtcttctcc
3481 ttaagtatag tgattacaaa tgaggaagga ggattggagg atttccggct gctttctgta
3541 gcattgaatg gggcctgctt tgttagatga cttcaattgc aggatatttc agaaggtgga
3601 tacatggact atactatatt tagactccag ctgccagtga gtctggggga ggcatactta
3661 ggcttgctgc aaaacgacga tccatggctt tcgtgaacaa gttatcctga aatatatgac
3721 aaaagatttt tttttaaaaa tcttcaggcc caactcagaa acatcccttta ctgataatat
3781 gcaaaggaga atttccagaa gaaaatgctt ctggatagag gagatggaaa atattgaaca
3841 gagttgttgg gagaagaaag tcctttttcaa cttctcacat tgctgccatc tgtcctagag
3901 acactctgag ctaccccccat aacatcatta aaaccacttc tctgggggtc ttgctctgtt
3961 gaacccaggc actgtatgag tcatttcctg aaaatattga acagactcat tgggagaaga
4021 caatcctttt cagcttctca cattgctgct cctgttccag agacactctg agctaccctt
4081 ataatgaaat gcgaaaagtt cccttgtcac tctcaaaggg catgcgatga gggagtggct
4141 cgctgctgct caaacctcta gggaagcata cagatgggca ggttgcggag ctccgacccc
4201 atggcagtgt ctaggggtga atatttacag ctcctgaagc cctagtgggc atgagttaca
4261 gggtgctctt ttagtttagc tgtctgtagg cggcttgtgt tagctcaagt agacccccctt
4321 tcttatcaca aggacagagg atttctgtat cccgggttct tgccttgatg tactggaaga
4381 atcacacgtg gacttggaga attagtgcaa ggtttatta agtagaagta gctctcagca
4441 gatgggggag ccagaaagga gaatggtttt ccccttgggt caggccactc ggcagcctgg
4501 gcggcctggg ctgtcctccg actgccctgg ccaaactcca aggcgttctg ctggtcagtg
4561 gcctgccagt gtgctggtgc ctgctgatgt gctcctctca acatccagct gcctgtgtgt
4621 tcctctgctg atgtggtcct ctccacgttc agccgcctgt gtatctgcct gcgagggtgt
4681 caggcacagg attagaggta tggcaggcca gggtggtctt gggaaatgca acatttgcgc
4741 aggaaaacaa aaatgcctgt cctcgcccag gtcattgggg tggagcccta gccagggacc
4801 acgacctcct cctctaccca gcatttccct tccccacttc cgtatcattt aaagggacca
4861 tgctcttccc ttcccagcac tcccatatca atagtatcat caaaaaccac ttctctgctg
4921 gtcgtactct gttgaacaca gacactgtat aagtcacttt ctaccaccaa tccagacagg
4981 cctcagtcat tgttgaacag catgaacaaa ttatcatgac tcactaaaaa gaaaaatata
5041 tcagccagta tgaaacagag aaaacagggc aaaaagagat ttatctgact catgagattg
5101 ttttagttgt tgttgctgat tagtaaggtt ttttaacacc aaatttttca gcatataatt
5161 gtctcgatct acttagaaca tcattaaagt caacataatt tgtccaggga ttgtaatttt
5221 atggtggaaa atgtccaaaa ttgcttatag ctattatcat taagtttta cctactcatt
5281 attccttagt gaatatgaaa tgggagaagt gttctcttat ccttctcaca gggtatgtga
5341 cggggtgtgg ctcacttctt tggtatcccg ctgctcaaac ctctaggggg agcatttttg
5401 gggctccaac tccatggcag tgtctagagt tgtttacagc tcctaaagcc ccagtgggcg
5461 tgtgttacag tgtgctcttt tagttttgcc atctgcaggc agcttgtgtt aatcagctca
5521 attagaccct ccaccttatt gcaaggacag agggttttct gtatcctggg ttccttccct
5581 agtgtattgg aaaaatcaga tcacacgtgg gcttggagaa tgagtgcatg agtgcaaggt
5641 tttattgagt cgtggaagta gctctcagca gatgggtggt gagccagaag gggaatggag
5701 tgtcttagcg tccagttgcc tgtgtgtgtg cccgctaggg tttcgggggt ttttacaggc
5761 acaggatggg gggcgtggca tttccctgcc cccctccctg tatcaaatag actgcagttt
5821 aaacataagg caataaatta aaaccagcat aaattgaaaa aaagtttaaa ttagttatgt
5881 ttctcccctg caaacttaaa aaacacacac acataaaatc actgtctttt ccaggaacat
5941 ggaagactag aaaatataaa attttttcac tacaggaggt tagaaatgct ggacaaaatg
6001 tagcaaacac tgtgttaagt gcataggtga gagttcagga aagtgaaata attcccaagt
6061 gacccaaaac aagagggaac tgaaaaacag cactaagaga gaaagaagga gcttctgtta
6121 ctaggaatca agatttgggt tatacctgct gctccctgat ggagacaaga ctttctgaga
6181 ctaagaaaat aaggactcag aattgaaatc cctacacaga accagatcat ttggagagct
6241 atacaatgat ggcaaggaga tcctagaaaa tactgcaaag ggagatgaca acacttatat
6301 taccatagtg gctttgggtg gggagaaata ttttgtgtaa aaggcagctt tcaagtgggt
6361 ttggtattta ttttagttac atggtatgag taccttcaag ctgagaaatt aactgaataa
6421 ctggttacaa attggcagta tttttgtgag gccagaaaga aacaaacaga agcaaaacat
6481 ttctaaagaa ttcacctaaa acccagacta cacaagattt ctataggtaa aacacttctt
6541 aaaatgaact tgtcttccaa aatacaaaac tcaagaagga acaatcttcc acgcacaaat

FIG. 7-4

```
6601 gttagcagaa gaagagatag aattatcagc tcaaatttcc aaataaggat aagatgtcaa
6661 agcaggaaat gaaaacggga taaaagaaaa tatgtatata tgtttttaaa atatacttta
6721 gaaaacaaag tagaattgaa gacgtgaaaa aaaacattaa aaataaaaat cattgaaagg
6781 gttaaacgtc agactgaaca caactgaaga gagaatttgt taactggata atggatttga
6841 agaagttacc cagaatgcac gagagaagag aggaggagag cagagagggg agagaggagg
6901 agagcagaga ggagagagaa gatgcaagag gactgaaggg ctgacataaa atctactaag
6961 agggccagac agaaagaaca gagaaaatgg aggattagtg ggcaatattt gaaggaaatt
7021 caatgaatga aaatctttgc caaattatag aaacacttaa atacttagat ttagaaaaga
7081 aagtaagttc caagctggag gaatgaaatt aatttagctt aaattaaaag caactaaaga
7141 taaaatgata atgattatct ataaaagaac atttattggt ataacagcaa ttatttatgt
7201 cagtagcagt aatacagata taaattaatg gaaacaatta ccagaatata gagtaaaaca
7261 tgccctttga ttttaaaaat aaatgctttt tgccaagaaa aaagatggaa tcatctcctg
7321 tcaaagaatg cttggcagta attcagtaaa ttaacagaat gtcctggaaa ctgaaagatg
7381 aacccagtaa ttctaaactg tatgtcatag ttattttcca ccaactaagg gtatatgatt
7441 gtatgaagga atgtggttgg gttaagggag gtgagtgggt atgcttattg aagaaatagc
7501 atgtgatgac aaagcacaat cccagatgtt gaagtcattt catctagagt ttggatattt
7561 agactatgtg aacccggtgg tggtcagtag tgtttaactt atattaggtt ctaatgcact
7621 aacctgtaaa ggctgtgaca taatgctctt agtgattaaa tagtttgtat ataaggctag
7681 gcgcgatggc ttttgcctat aaccccagca ctttgggagg ccaaggcagg ccgaggtggg
7741 cagattgctt gagctcagga gttcaagacc agcctagcca acatggcaac aacccatctc
7801 tacaaaaacc tacaaaaatt agctgggcat gatggcaggt gctgtagtct cagctactca
7861 ggaggctgaa gcgagaggat ggcttgagcc tgggaggctg aggttgcagt gagctaagat
7921 agcaccactg cactgcagcc tgtgtgaaag agtcagagcc tgtctcaaaa acaacaacaa
7981 aaaaaattat ttgtatgtaa taaaccaaag ctagcaatgt gtttcacata ctttccttaa
8041 atttcaggga tatcctttct cataaataat acctgtgtgg gtgttttttt tttactacaa
8101 aagtaattcc tggtggtcat agaaaatctg aaagacatat ataagtcaca agaaaaaatt
8161 ttaaatttcc tttactctta ccacccagag aaaatattgg tcagcctttt aggacatttt
8221 aagttgattt taattttttt aattcaactt tgtatttcaa gataattgct aacatgcaag
8281 tctcaaataa tagactgatc ttgggcaccc tttacccagt ttctcccaat ggtaacatct
8341 ttagtacctt tagtatagaa aaactatagt acattcaatg gtaggctgtc acagaaaaaa
8401 aaggaaaaga aaagctatag tacaatatca cagccatgat attaacattg atatagtcaa
8461 gatacagaac atttcattca ccacaaggat ccctgaagtt gttctttgat agtcacactc
8521 actttccttc ttccctcact ccttccttaa tcctggtaac cactgattct ttctccatgt
8581 ctatactttc atcatttcaa taatgttata taaaatggaa tcatacagta tgtaacattt
8641 tgggattgac tttttgttt agcataactc tgtggagatt cacctagatt gttgcctgta
8701 tcaatatttt tattccattc tattgctaaa tagtgttgta tggtatggat atgtgtaacc
8761 attcacttgt tggaggacat ttgtgtgttt ccagtttttg attacggtaa gtaaagttgc
8821 tataaatatt catgttcagg ttttttgtgt taaatgtgtt aatttctatg agataaatgc
8881 ccagaagtga aattgctggg tcatatggta gttgcatgtt tagtttctta acaagctcca
8941 acttcatatc attacatttg aagtaagttt cttatatagt agatcatgtt tttaaatccg
9001 ctctgacaat ctctgtcttt taatttgtgt agttagaata gtgttatgac ttaacactga
9061 catttttgt tgtttcctc tatttgtttt ctctagtttt tgttttagtt ttccttttct
9121 tgcttcccta caggttactt gaatatttta aaactttatt ttaatttagg tacagtgttt
9181 ttgagcatat gtctttgtat agcttttta gtgatattaa attatacttg atattacatt
9241 atatatacat aacttatcac agtctattgt gtcattattt tgaatgaaat atggaagtct
9301 catctcttta tattcccttc cccttcccca tttatagtat aattattata actatttcct
9361 gcccatatat ttggaaccac atcaatcagt gttataactt ttgcttaatc tggtaaacat
9421 aacaacaaaa tttaagaaga aaaggaaaac taattacatt tatgcatatt tttgtgtact
9481 atctttattc cttcttgctg ttttgaggct ccttctttca tcattccttt tctgtttaga
9541 aaaattcctt tagcctaaaa ggagtaggtc tgctagtgac aaattatctt agtttccctt
9601 taactgagaa tgtcttaatt ttcccttcag ttctgaagta tatttttact gggtatagga
9661 ttctgggttg acaattcttt tcttttagca tttaaaaaaa atattgtgcc acttccttcc
9721 tgccgtgaat aagacattta tattgctgat gttaaggaat aagagaaata tccaatgttg
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FIG. 7-5

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 9781 aaagagagtg caagagaatg tatatagtcc atatagaatt aaatctgtgt catggtgggg
 9841 tactctataa tggttttgct gttcagtata gagcttgggc attctcaaat ttaatcttca
 9901 tgcattaagc tattcaaaac caactacaaa catgcttgat atgtagtgtg ttatatttct
 9961 aaggcatata tttaccttgt ggtgttatga atttgttgtg taagaggcat actaagctaa
10021 taagtaagcc tagatgccca ttcagtgtag aatctatgtg gcctgaaaag ggagagaaag
10081 attaatggta agtattaaaa taactgtgct aagcataaaa atagctaaca ttcatttaac
10141 actttccatg tgccagatag tatggcattc atttaacagg tgttaactat ttttattctc
10201 acaatacatt gtttgaggca gataatacta atttccccac tttatagatg agaaaactaa
10261 agctcgggat gttatgtaac ttgcccaagc cacacataat aacagtggca taaccagaat
10321 tcagaattta aatccataca gtctgtcttt gattccatgc tgtctatcct gtttaacttt
10381 tacatgacaa agatatcttt tacagcatta aattgcacca gggaactcta acccatctgg
10441 cccgggtcag aaaagggaca aaatgtcaac ttttaagtat tcaaaaatgg tatttgtttc
10501 tttcatttat tcatttgcta tgtagcaatc accattgtat aatatctcca taagaagata
10561 ttgtttgaaa ggaaatccat catttattaa aaatatgtat aaaacactta caatgggcca
10621 ggcattgttc taagtagata agatacataa attaccaaaa cacacaaaga ttcacaccat
10681 taaagagttt gtatcttagc atgagggagg aagaaaataa acaatagcta aaaaaaaaga
10741 aataaattat atagtatatt tgaaagtgaa gatgaaaaaa ataaaactag tgtaggtaag
10801 tgtgatcatc agagaagcaa gcaacattaa atggggtggt cactattgga agatgacata
10861 tgatgatgag agaatttgtc tttgaggaag attccagcta gagaagacaa ttctatatcc
10921 ctaaattggg aaagcacatg catgcctgaa gactggcaag agggctagtg tatggaagta
10981 aatgatcccg tgaaagagta gtaggaaata aagtaaaaga gacatgtata atcatgaaaa
11041 tcactgaaag gtttggagat attgtggggg gagtccttgt ttgttggttt tttaatagca
11101 gtgatataat tcacatatca taaagtttat aaagtataca attcagtagt ttatatatat
11161 tcacacaggt gtgctaccag tatcacagtc caactccaga gtatttccat cgctctccaa
11221 aatcaccata tctattagca accactcttc aagtccgcat cccccagcct ttggaaacca
11281 cagatttcct caccgcctct aagaaattgc ctattttgag gctcaaagta aagggttgga
11341 ggaagatcta ccaaccaaat ggaaaacaaa aaaaggcagg ggttgcaatc ctagtctctc
11401 ataaaacaga ctttaaacca acaaagatca aaagagacaa agaaggccat tacataatgg
11461 taaagggatc aattcaacaa gaagagctaa ctatcctaaa tatatatgca cccaatacag
11521 gagcactcag attcataaag caagtcctta gagacctaca aagagactta gactcccaca
11581 cagtaataat gggagacttt aacaccccac tatcaacatt agacagatca atgagacaaa
11641 gttaacaagg atatccagga attgaattca gctctgcacc aagcagacct aatagacatc
11701 tacagaactc tccaccccaa atcaacagaa tatacattct tctcagcacc acactgcacc
11761 tattccaaaa ttgaccacat agttggaagt aaagcactcc tcagcaaatg taaaagaaca
11821 gaaattataa taaactgtct ctcagaccac agtgcaatca aactagaact caggattaag
11881 aaactcacac aaaaccgctc aactacatgt aaactgaaga acctgctcct gaatgactac
11941 tgggtacata acgaaatgaa ggcagaaata aaaatgttct ttgaaaccaa cgagaacaaa
12001 gacacaacat accagaatct ctgtgacaca tttaaaacag tgtgtagagg gaaatttata
12061 gcgctgaatg cccacaagag aaagcaggaa agatctaaaa ttgacaccct aacatcacaa
12121 ttaaaagaac tagagaagga agagcaaaca cattcaaaag ctagcagaag gcaagaaata
12181 accaagatgg gagcagaact gaaggagata gagacacaaa aaacccatca aaaaaatcaa
12241 tgaatccagg agctggtttt ttgaaaagat caacaaaatt gatggaccac tagcaagact
12301 gataaggaag aaaagagaga agaatcaaat agatgaaata aaaactgata aagggagtat
12361 caagaccgat cccacagaaa tacaaactac catcagagaa tactataaac acctctacgc
12421 aaataaacta gaaaatctag aagaaatgga taaattcctc gacacataca ccctcccaag
12481 actaaaccag gaagaagttg aatctctgaa tagaccaata acaggctctg aaattgaggc
12541 aataattaat agcttaccaa ccaaaaaaca gtccaggacc agacagattc acagccgaat
12601 tctaccagag gtacaaagag gagctggtac cattccttct gaaactattc caatcaacag
12661 aaaaagaggg aatcctccct aactcatttt atgaggccag catcatcctg ataccaaagc
12721 ctggcagaga cacaacaaaa aaagagaatt ttagaccaat attcctggtg aacatcgatg
12781 caaaaatcct caataaaata ctggcaaacc gaatccagca gcacatccaa aagcttaatc
12841 accatgatca agtgggcttc atccctggga tgcaaggctg gttcaacata cacaaatcaa
12901 taaacgtaat ccaccatata aacataacca aagacaaaaa ccacatgatt atctcaatag
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FIG. 7-6

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12961 atgcagaaaa gaccttcgat aaaattgaac agcgcttcat gctaaaaact ctccataagc
13021 taggtattgt tgggatgtat ctcaaaataa taagagctat ttatgacaaa cccacagcca
13081 gtatcatact gaatgggcaa aaactggaag cattctcttt gaaaactggc acaagacagg
13141 ggtgccctct ctcaccactc ctattcaaca tagtgttgga agttctggcc agggcaatca
13201 ggcaggagaa agatataaag ggtattcact taggaaaaga agaagtcaaa ttgtccctgt
13261 ttgcagataa cctgattgta tatctagaaa accccatcgt ctcagtccaa aatctcgtta
13321 agctgataag caacttcagc aaagtctcag gatacaaaat caatgtacaa aaatcacaag
13381 cattcttata caccaataac agacagagag ccaaatcatg agtgaactcc cattcacaat
13441 tgcttcaaag agaataaaat acctaggaat ccaacttaca agggttgtga aggacctctt
13501 caaggagaac tacaaaccac tgctcaacga aataaaagag gacacaaaca aatagaagaa
13561 cattccatgc tcatggatag gaagaatcaa tatcgtgaaa atggccatac tgcccaaggt
13621 aatttataaa ttcaatgcca tccccatcaa gctaccaatg actttcttca cagaattgga
13681 aaaattactt taaagttcat atggaaccaa aaaagagcct gcaccgccaa gtcaatccta
13741 agccaaaaga acaaagctgg aggcatcacg ctacctgact tcaaactata ttacaaggct
13801 acagtaacca aaacagcatg gtactggtac caaaacagag atatagacca atggaacaga
13861 atagagccca tggaaataat aacaaacatc tacaaccatc tgatctttga caaacctgac
13921 aaaaacaaga aatgggaaag gattccctat ttaataaatg gtgctgggaa aactggctag
13981 ccatatgcag aaagctgaaa ctggacccct tccttacacc ttatacaaaa attaattcag
14041 aatggattaa agatttaaat gttagaccta aaaccataga aaccctagat gaaaacctag
14101 gcaatacaat tcaggacata ggcatgggca aggacttcat gtctaaaaca ccaaaagcaa
14161 tggcaacaaa agccaaaatt gacaaatggg atctaattaa actaaagagc ttctgcacag
14221 cgaaagaaac taccaaccta cagtgaacag gcaacctaca gaatgggaga aaatttttgc
14281 aatctactca tctgacaaag ggctaatatc cagaatccac aacgaactca aacaaattta
14341 caagaaaaaa tcgaacaacc ccatcaaaaa gtgggcaaag gatatgaaca gacacttctc
14401 aaaagaagac atttatgcag ccaacagaca tatgaaaaaa tgctcatcat cactggccat
14461 cagagaaatg caaatcaaaa tcacaatgag ataccatctc acaccagtta gaatggcgat
14521 cattaaaaag tcaggaaaca ataggtgctg gagaggatgt ggagaaatag gaacactttt
14581 acactgttgg tgggactgta aactagttca accattgtgg aagacagtgt ggcgattcct
14641 caaggatcta gaactagaaa tatcatttga cgcagccatc caattactgg gtatatatcc
14701 aaaggattat aaatcatgct gttatacaga cacatgcaca catatgttta ttgtggcact
14761 attcacaata gcaaagactt ggaaccgacc caaatgtcca tcaatgatag acctgataaa
14821 gaaaatgttg tacatataca ccatggaata ctatgcagcc ataaaaaagg atgagttcat
14881 gtcctttgta gggacatgga tgaagctgga aaccatcatt ctcagcaaac tatcacaagg
14941 acagaaaacc aaacaccgcg tgttctcact cataggtggg aattgagcaa tgacaactct
15001 tggccacagg aaggggaaca tcacacaccg gggcctctca tggggtgggg gtgggggggag
15061 ggttagcatt tggagacata cctaatgtaa atgacgagtt aatgggtgca gcacacacca
15121 acatggcaca tgtacacata tgtaacaaac ctgcacattg tgcacatgta ccctggaact
15181 taaagtataa taataaaaaa aaaataataa acaaatggta tccctgggaa tcatatagat
15241 aatatggtaa ataaaatgga aaaatagaaa aaaaaagaaa ttgcctattt tggacatttt
15301 atatgaaaag agtcatataa tatatggcct tctgtgtctg gcttcttcca tctgacataa
15361 cgttctcaag gtacatccat agtgtagcac atatcaatac tttgttttct ttccttcctt
15421 ccttccttcc ttccttcctt ccttccttcc ttcctttctt tctttctttc tttctttctt
15481 tcctttcttt cttttctttc ggcagagtct tgccctctca cccagcctgg agtgcagtgg
15541 tgggatcttg gctcactgca agctccacct ctgcctcctg ggttcatgcc attctcctgc
15601 ctcagcctct tgtgtagctg ggactacagg caccccgccac cacgcccggc taattttttt
15661 ttgtattttt agtagagatg ggtttcacc atgttagcca ggatggtctc aatctcctga
15721 cctcatgatc cacccacctc ggcctcccaa agtgctggga ttacaggtgt cagccacccg
15781 tgcctggcca ctttgttcct tttcatggct gaatgatatt tcattttatg gttataccgc
15841 attttgttta ttcatttgtc acctggattg tttccacttt ttggctgtta caaataattc
15901 tgttataaac cttcttgtac aagttttggc ataggtatat ttttcatttc tcttgagtga
15961 gaacctagga gtggaattac tgggctatat ggtaactcca cataagtttt agaattttac
16021 tctgagtata cttatttcaa taagtataaa gaggagtgat ataatctgac ttgcacttta
16081 aaagataact cagactatgg tgttgagaat atactgtaag ataacaattt tttaaaaagc
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FIG. 7-7

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16141 ggagcagccc agcatggcag ctcacatctg taatcccaac actttgggag gccgacacag
16201 gaggatcact tgagcccagg agtctgagac cagtctgggc aacatagaca aaccctctat
16261 gtctctacaa aaaaatacaa aaattagcca agtatgatga tatgtgcctg tagtcccagc
16321 tatttgagag gctgagatgg aaggatcact tgagtccagg aggttgaggt tgaactgtga
16381 tgacaccaca gcactccagc ctcagtgaca aggcacgact gtgtttcgaa agacagagag
16441 aaggagagag agggagggag ggagggaggg aggaaggaaa ggaaggaagg aaggaaggaa
16501 ggaaggaagg aaggaaggag ggagggaagg aaggaaaata acctagttaa aagtttgtac
16561 ttggttgaat agcttccctc caaaattcat gtatacctgg aaactgaatg tggcctcatt
16621 tggaaatagg ttctttgcag atgtaatcag ttaagatgag gtcataccgg gttgaggtgg
16681 accctaatct aatgattggt ttacttataa gaaatgagac attttagctg ggcatggtgg
16741 ctcacgtctg aaatcccagc actttaggag gccgagacgg gtggatcact tgatgtcagg
16801 agttcgagac aggcctggcc aatatggtga aaccccatct ctactagaaa tacaaaaatt
16861 agctgggcat ggtggcaggc acttgtaatc ctagctactc cagaggctga gtcaggagaa
16921 tcacttgaac ccaggagatg gaggttgcag tgagccgaga tcatgccact gcactccagc
16981 ctgggtgaca gtgagaatcc atctcaaaaa aaggaatgag acatctggac acaaacacag
17041 gaaaaaaaaa atccatgtga agjtggacac atagattgaa atgttgcatc tacaagccaa
17101 gaaatccctg atgtcactga cactagatgt accagatcta gaaaagaaga acctacatcg
17161 ctcctggatc cttcgtagag agcaaggcac tgctaacacc tttattttgg acttctatcc
17221 tccagaactg tgaaagaaaa aaaaatctgt tgctaaaagt cccccaactt gcagtacttt
17281 gttacggcag cccgaggaaa tgaatacgga ggatattgtt gttatttagg tgagaaatag
17341 agttggccca gactagtgtg atagaagtca aggtgaagaa aaaaggtcag aatctagaat
17401 tattttgaaa gtagagctat aagatttacc aaaggattga atgtaggttt tgagggaata
17461 ataaaggaac caagaataat ccaagacttt taatctaaac tattggaaag atgttgccat
17521 aaactgaatg aggaaggctt tgggtggagc agcactcagg ggaaatacaa agagtccagt
17581 tgttaacttt tagaagccta tttaataccc aagtggagat gtcaggtagg cagttgtaca
17641 taaaagtctt gaattctgga gagaggtctc agctaaagat ataaatttct gagtggtcca
17701 cataacagag gcattttcag ccattagatg gcacaaggtt gaaagagatc actaaggaat
17761 gcagagcaga gacgaaagca aggcctaccc tgggtactgc aatgttagta ggtcaaggaa
17821 aagagaagaa atcagcaaag gagactagaa gggagcaact ggtgaggaaa aaggaagact
17881 aagaaattgt gatgcttgtc actaatcatc agggaaatgc aaattaaacc acaaagagtt
17941 accagcttac tcctgcagga atggccataa ttaaaaagtc aaaaagcaat agatgttggc
18001 atggatgtgg tgcaaaggga acatctttac attgctagtg ggaatgtaaa ttagtaccac
18061 cactatagaa agtaatatgc agattcctta aagaactaaa agtagaacta ccattcaatc
18121 cagtaatccc actactgagt atctacccaa aggaaaagaa gtcatataaa aaagacacat
18181 gtacatgtat gtttatagca gcacaattag caaatgcaag aatatggaac caatctaagt
18241 gcccatcgac cgagttgata ataaaaatat ggtatatatg tatatgccat ggaatactac
18301 tgagctataa aaaggaacaa taataatgtc ttttgcggca acttggatag agctggaggt
18361 cattattgta agtgaagtaa ctcaggaatg gaaaacccaa tattgtatgt tttcacttac
18421 aagtggaact tagctataag gatgcaaagg cataagagtg attaatgggc cgggcgcggt
18481 ggctccgcct gtaatcccag cactttggga ggccgaggcg ggcggatcac gaggtcagga
18541 gatcgagacc atcccggcta aaaccgtgaa accccgtctc tactaaaaat acaaaaaatt
18601 agccgggcgt agtggcgggc gcctgtagtc ccagctactt gggaggctga ggcaggagaa
18661 tggcgtgaac ccgggaggcg gagcttgcag tgagcagaga tcccgccact gcactccagc
18721 ctgggcgaca gaacgagact ccgtctcaaa aaaaaaaaaa aaaaaaaaaa aagagtgatt
18781 aatggacttt gggggctcga tgggggaggt ttggaggggt taagggataa gagaatattg
18841 ggtacagtat aaactgcttg ggtgacaggc acactaaaat ttcagaaatt agcactaaag
18901 aacttaccca tttaaccaaa aaccatctgt acccccaaaa actattgaaa ttaaaaaaaa
18961 attgtgattg atgtggtttc gctgcatcct cgcccaaatc tcatttgtat tttagcccca
19021 ataatcccca catgtcatgg aagggacctg gtggcaggta attgaatcat ggaggtgggt
19081 ctttctcgtg ctgttctcgt gatagtgaat aaatctcaca agatctgatg gttttataaa
19141 ggggagttcc cctgcacatg ccctctttgc ctgctgccag gtaagatgtg tctttgctcc
19201 tctttgcctt ctgccatgat tgtgaggcct cttcaaccat gtgaactgtg agtcaattaa
19261 ctcttccctt tataaattac ccagtcttgg gggtgtcttt attagcagcg tgagaacaga
```

FIG. 7-8

19321 ctaataaaat gatacctgta ctagaatcca cttgaagaaa gggaagcaca aattgatcac
19381 caaggtgtga cataggaaag catagtttga tggtccatca aacactattg ataggaaaaa
19441 gtgagaacta agaattgagc attcaattta gcaatataaa tattattgtg actaggatga
19501 acaatagttt cagcagagtg tttaaagtaa aatcttggtt agtgggtttg tagctgaaag
19561 agaggaaatt ggagacagca attacagaca cttgaaattt ttatcaggga agatttcaag
19621 catataaaaa tatgaattaa caaaaaatga aacccatcac ccacattcaa ttgtttttaa
19681 ctgatagcca atgttgttca tctacttccc cactccctct gtccctacct taccagatta
19741 ttttgaagat actctgaaaa aataatttaa cttcattcat aaaaatttag acttctgctt
19801 taaggctctt gcatttttc ttttctata aaaggagcaa aaaaacagat ggtaatttgc
19861 aggtggaagt gaggttaaga gaagatattt gtaaaccact tttggatccc tgggttcaga
19921 tgtggactag caagagagtt ggatttaacc agggttttgg ttttgccatg tataaaacac
19981 tgtgaggttc aagggtgtat gcaaaggaat gattactatc ctttgataat tgactatgtg
20041 accataagca ttttagctgg gtaaggagtg gagtgatgac ataaagtggg tgaaatacac
20101 tgacaaggtg atagaatcat agcttagagg tcaaggtgga gtaataaagt ggtggtggtc
20161 aaagagcaca agttagtcag agaagcgggt ttacaggaaa taagccagag aatgttcatc
20221 aacgtgtata ttaacccatt tcctgtttag aaaaaaaaag tgtggcttgc tgccagcact
20281 cactgaattt tacgtaaaca cactctttga ggctgaagca aatctgactg atttttcaat
20341 gtgaaaataa aatataaaaa ttcttcatgg agttatttct aaacagaact tgtctctaat
20401 cctaatgtaa cagaaatgta tatgatatta cattaggatt agagacaaga gaacaagaat
20461 attcttgggg caaacaggaa atgggttaag gaattagact agagtagatt tatagataag
20521 gagactaagc tcaagagcga aaatcataga gaaatgagtt gaattacaga atgggagtgg
20581 ctggtgtctt ttagaaagga gggagtatat tctgaacata gcaatgagga tcaagaaaaa
20641 attagcccat gaccagagag agctgtgaga gtataaataa acagtgccca cctaagaaga
20701 ctgtaagata agcaggaggg agagctgtaa ttccattaga gcaatgaaaa gagattttcc
20761 ttggccactg cgtgacgctt ttttaacatg ttttttccc tcaaccataa acaattgcag
20821 gcacagttgt gagcatgggc aaattcaggt tttatgtgac ctgaaactta cttagtttgg
20881 aggactttct ttaaggaaag actgtaaaat ttttattttc ccagatttta ccaacaaaaa
20941 agtgcaatta tgcaaaccca ttatccactt accttccaat agcccactcg aaaggggcta
21001 ttgggaaaca attctctatg gatattttca cctttctgca tgatccaggc tttctgagca
21061 aacaatatgg tcaaacttga ttaaataatt attaagtggt aaacatgcct tggaagccag
21121 aaattgtgta ttaatctgga aatatttatc ccttggataa atcccacttg atcatgctaa
21181 atgatctttt taatgtgttg ttgaatttgg tttgctagca ttttgttgag gatttttaca
21241 actatgttca ccagaaatat tggcctaaca ctcttttttt ctgtgtcttt gtccaatttt
21301 ggtatcaagg taatgctgac cttgtagaaa gagtttggaa gtattaccac ttctcccatt
21361 tttttggaa gagtttgagt tttttcttta aaagtttgac agaattaagc attgatgcaa
21421 tcaggtccta ggcttttctt taatgggaga ctttttatta cagctttgat ctcattactt
21481 gtttgttcaa gttatttttt tctttatgat tcaatcttag taagttgtat gtgttcagta
21541 atttatcaat gtcttttaga ttttccaaaa gaaacttgtg gtttcttttg gaaaaagttg
21601 tccataattg tttttaatga ttctttgtat ttctgtggtc tcatttgtta actctccctt
21661 tttgtttctg atttttattta catgggtctt ttctcttttg ttcttagttt aggtaaaggt
21721 ttgttaattt attttttcaa aaaaaccttt ttgttgatat tttgtatttt ttactctcaa
21781 tttcatttac ttctgctctg atctttatta tttctttctt tctactaaat ttggttttgt
21841 tttgttctct tttctagctc cctgaggtgc atcattaggt tgttaatttg aagttcaaag
21901 ataatataga aatacagtgg tcattgtaag aaagttttaa cctagacaaa actgttcatt
21961 tatgaagtgg attggaaaag aagaaattct gaattcctca agaacaatgg aatgtagatt
22021 ttgattatca tgcagagcct tacaagagac tcaataaatc aaaatatgcc tctcttaaag
22081 acttctggcc agaagcagtg gctcacacct gtaatcctag cactttgtga ggctgaagag
22141 ggcagatggc ttgagctcag gagtttgaga ccagcctgga caacataggg aaacctcatc
22201 tctattaaaa ataaacataa taataacaat tagccaggca tggtggcaca agcctgcagt
22261 cccaactact caggaggctg aggtgggagt gttggagtag gtagttaggc agacatgaac
22321 atcagaagaa aagcccatcc cccaccagta atgtcaggca accattaggt gatggtcagg
22381 tagttgttaa gcagtctttt taaaataata attggttgca gtcagtgcca gagaaaggca
22441 gtctcccaat atatggaaaa cacttgaagc cggtggccaa cagcttccca ataaggagct

FIG. 7-9

22501 gagcaagcag tctcaagcat gcactaagag gcaaaatggc agagtttaac tggtgtatga
22561 ccagttcctc taggaacact tgactggcaa gggaaaaacg cctcaagtga gcatgcacac
22621 aacttcagta aatacactgt gcatgtggcc gctcacaagt gctggcaggc cactgtgcat
22681 gtggatggcc caccccaagg aaaaatcaag agtggagaga cacaaaactc cagaagcatg
22741 ccaacatata aaaccccaag tcaaaggtca aacagggcac ttggatttct caggttgcca
22801 acttggccct cttccaagtt actttacttt ctttcattcc tgctctaaga gtttttaata
22861 aactcttact tctgctcaaa aacttgcctt ggtctctcac tctcgcttat gcccctcaga
22921 caaattcttt cctttgaaga ggcaagaaac aagttcttgc agacccatac agattcactg
22981 ctgctaacat actttgatgc cacatggctt ggatacgttc cccagtggta agaaatctct
23041 atgcctctcc ttctttggct ggagaagaag gagatctgta cacagttttc ttctcccctt
23101 tcactctcct gccttccaac caattcccag aactattact ctcagccaca gtggctctgc
23161 acccgctggc tgatctctca gcttaccctg accggtggct tacaggggta ggaaggacct
23221 tggagtccac accatgtaga cctgaaatac taatggccct cctagacagg aggctcatga
23281 gagtggtagg gctaaagcct aacactgtgc aatgtctgca atttcctctg ctttttcaaa
23341 tacaattgtc tttttcccca aaacccacac cacctattct cctgtttttct ctgtgtgtgt
23401 tctgaaacgg ccttgtgtgc ccaccacact gtctacctca ggggcaagtc tgtctctctt
23461 ctttcacttt gtatgccacg caacttcctt ctctacgtta aacacacact ccctgttatt
23521 cgttcaccca tggctcttgt tgcatttgta cagcagcaga gaaacaggct cccttgcaga
23581 tgtccattgg ctcattgcca ggatagacac taattagaac cccagttctt ccagctcctt
23641 atgacttacc atacactttt catccctgtt atgctccagg gtcaagtttt tggtggcttt
23701 tgaagcagtt tttatggctg cataggacat cactctgtgg ttctttaagg atctcaccta
23761 cttgcttttt ttgagttagc accccctttg ggaggagaga aaattcttcc tttgccattt
23821 gtgggctctt atcccaagct ccgagttctc caaagattcc tctttatgtc aagagggcaa
23881 ataaacattg ctgttttgaa tcccagggct gctgtttatg tgagcatatg aaggttttcc
23941 gtcagtattc ctcatgcttc ctcccacttt ctcctatagc agaggggttg tcctgtctac
24001 tcaagcattt cttctggata ttaccccagg aggacaggga acctcaaata caaaattttc
24061 tccattcctc taatcacttc catacсctcc tcaatgtgct taaggactct caaggtcata
24121 tttaaaggga gggaagtgca gcctcttgca gcagttggct gaaaaacaag cttctcatct
24181 acttaaagaa catgggaaat ggattataaa aaaaaaagag gtaatcattt tgttgccaga
24241 atgctgtgag taagagtcac tataagatca tggagataag gatataggcc agcccaaggc
24301 tgcaggtgca agagacaaag tacccatagg acagagagag ataaaggctg gtcacaggcc
24361 acaggcacag aacagattac cattagaaca gagatgaagg caagcttagg ggtacctggt
24421 aagaccggtt tattctgaaa ctccaaggat aaataggggа cccctgttca cttgggtatt
24481 tcctctgttc tcaagtgggt aattgtgatg agatgggaca caggtttggg tacccagtaa
24541 gacaggttca ttctggaacc ctaaggatga atgggggatg ccctgttcag gaaaggataa
24601 tagggaaata aaaggagata ccttcttttt cctttttttc tcctttgttg tatcttcaca
24661 gatgggtaat cacatctccc tactacagta caaacccttt ggatgcatcc tcaagaactg
24721 aaagtttgac cccaaaaccc tgaaaaggaa aaggctaata tttttctgta tcacagcctg
24781 gttttaatac aagctccagg agcaggaatc ttgaccagaa agtggaagca taaattttaa
24841 atttttaata tgctctatca gctagatctg ttttgccact agcagggaaa atagacagaa
24901 atcccctatg tacaggccct aagaaacaac ccagaccttt gttgggcttg taaaattgat
24961 ccagtgatga tagcagccat aatcatgctg ccccatctgg ttggttcggg agacccctca
25021 ttgggtttac ccagggctca agccctggag gaaccaagag tggagcttag ctcaaaccct
25081 accctttggc cccattatat ccaagtctcc tggccctgga gccttcccctt ccctacccag
25141 ggccatcctc tggacaccac cccctcaagt tatgctccct tcaagaagtt agttgaccta
25201 ctagagttca gactccattt aatatgcaag acctgagtca aattaaaaca gaactaggga
25261 agtttatgga ggatcctgac aaatgcatca aagggttcca taaactgggc ttaacatttg
25321 aactcacttg gagggatctc tcagtcatac tgggacaaac cctgtctaag ggagaacatg
25381 actccattat ggaggcaccc caaaaatttg caaatgcaat acacatgact gaccttggtg
25441 gctaccctgt atgggccacc acagttcact gggtcagccc caattgggat tacaataccc
25501 atgggggcat atgggcaaag aattgcatgt tcttatgtct cgtagaagga atgaaagcta
25561 gaagagccaa gcctgtaaac tataataaaa ttggtcttaa tagatcaagg acctcttgga
25621 aaccccacca ctttcctaga gaggctacaa gaggccctgg tgaaacacac taacctagac

FIG. 7-10

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25681 ccagagacat cacaagggca actggtcctg aaggaccatt ttttaactca ggcagcccca
25741 catatttgga ggaaactcta aaaactagca ctggacccca aaaccottat gcctgacatc
25801 ttcaacatag cttcctctgt cttttacaac caggactagg agaaagagga aagggctcag
25861 ggaaagaaga ggtaaaagga aaagcagcag ctccaactat tggctgccct aaaaatccac
25921 cagccctctc caagttgccc tcagaatact ctcccagtta actgccatca gtgtgggaag
25981 ccaagacact ggaagacaaa ctgccgcagt gggacagatg ggaaaaatcc ctacatggct
26041 tgccctttct gtcacaaact caaccactgg aaatggaact gccctgaggg ctgaagggcc
26101 cctagaacag aatcccaatt cctgatggcc ttaagctgaa aaggcccgcc actccagcag
26161 ctcctggact gaacattact attgaaggga cagagctaag ggctgctttg gatgtggcag
26221 gtaggactat aagttttctt tgggaaatgg ccttcttggt gcttatgtct tcatgggcaa
26281 ttatcctcca aggtgatggg gttaaattgg gtgcccataa cccaaaggtt cactcctcat
26341 ctttgctgcc tgtgagggga gactgtcttt tctcattcat ttttaatgat agcagaatgt
26401 cctacactgc ttttgggtag agatatttta tttagactag gggcttagtt aaccttttcc
26461 aaatgcaatc ttatgcctca agcaagtcac acacaaggat gaacttccca gacttttcca
26521 ttaatccaga agtctggggc tcaagaatat caggaaaggt cataatgata atgcctatag
26581 taactcagct catggatcct tctagctaac cttgtagaag gcagcttcct ctttgacctc
26641 tggccaaaag ggacttcatc ccctaattga aaaatgtcta aaacatggat tattaatata
26701 ctataactcg ctatgtaaca cccctgtctt acctgttaaa aagagcaatg gagcaatgga
26761 caatataggc tagtccagga cctgcaaatc ataaatgagg cagtagtccc aatacaccca
26821 atggtcccca acccatatat tattctagga gaagtacccc cagatgctca tttgttctca
26881 gtcttagagc tcaaagatgc tttcttttgt gtctccctag actcatcctc ccaatttgta
26941 tttgcacttg aattggggaa tgaaaaagga aggagtctac agctaatctg gatagtactt
27001 ccgcaaggtt ccagagatgg ttccaatttg tttgggcaag tcttggctag agatttgcag
27061 gacctaactc ttgagggttg agggtgtctc ctacagtaca tagatgacag atgacctgtt
27121 aatctgctcc cctacaagag agttatgaat cctgcatcta gtccagacac tagattttct
27181 cacagagggt acaagatgtc taaggccaag gcacatcgat taagaaaaga ggtttaatac
27241 ctggggataa tcctgatccc acagagaaca taagctttct ccagaatgga tacaggccat
27301 ccttagaata cctaccccaa ccacctaaaa gcaactttgg gcttttctgg ggatcacaag
27361 atattccaga ctttcaatac tggcatatgg tggaattatt aagtccttat atcaggttct
27421 aaaagaaggg actcatcagg acccacttct ctgggaaaaa gatcagaagc aagcctttag
27481 agaactaaaa actgccttct cacaagccct agcccttggg ctacccatac taaccaaacc
27541 gtttcagctt ttcatcactg aaagtaagct gtagccctgg aagttctaac tcaaaccatc
27601 aggccatcaa atgtcctata ggttactttt taaagaacct agacccagta gtgcaagggt
27661 ggccacactg cctaaaggta gtggctgcag cagccctttt acccaaggag gccctcaaaa
27721 tcacaatggg acagtcggtc aggctcctga cattccaact aataggcccc ttattggata
27781 taaaaagacc gcaatggatc actgacaaca gactgctaaa ctaccaagtc ttgttgtcag
27841 aaaacccaca ggtaacagtt gagtggtgtt ccactcttaa cccagcctcc ttactgccac
27901 tactagaaga ggataactga acacattcat gttgtgagat acttaaccaa atttatgcca
27961 gccaggaaga cttacaatat cagcccctag gttatctaga tgaaatatgg ttttcagata
28021 ggattggctt tgtcaagaat ggagatagat atgcagggta tgctatatgt cccttcaccc
28081 agttacagag gtggtagctc tgtccctggg gacctcagtg caactagctg acccatcaca
28141 ttgaccaggg ccctaaaact tggggaaaga aaaagaataa ccacatatac agattccaaa
28201 tatgccttcc tggtgcttca cacccacatg gctaaccaga aggaaggggg atacctaaca
28261 gcttggaata ctcctattac atagagacct ccaatcttag agctactaga ggctgttcat
28321 ttgcctcagg aagtggcagc agtgcactgt aaagaatacc acaggggttc tgatgaaact
28381 gcatggggaa atatgttatc tgaccaaaaa gctaaagagg cagccatctc aaaagacacc
28441 tctgtgggga ctttactccc ctctctcccc agtgaactgc ccttctccaa tacactaagg
28501 aagaaataga ttgggccatc cagcatgggt ataaagaaga aaccaatgga tggtacatgt
28561 tgggagaact tctccatcta cctaatgctt cccagtggaa agtcatcaag tgtttacttg
28621 actcatgcca ccttgaaaag gacagtctag ggtaaatttg taaatgggtg ttcagtggga
28681 agggactaac caaaattatt caataggttt gtcaagcctg caccttgtga accacaaata
28741 atccctagat ggggaagccc cctccataat aagtcaaatc caaaggagag gtacatactc
28801 tggggaggac tggtagatgg acttcactca gctacccaca tgccacaggt gtaagtacct
```

FIG. 7-11

28861 cttggtctgt gtaggtacct tcacagaacg ggaagactcc tgccccacaa ggactgaaaa
28921 ggcacaggag atagctgact tgctcctaaa ggaactcatt ccctgctttg aactccccag
28981 gtcattgcag agtgacagtg attcatcttt ttttccccaa gtaacttaac aggttagtaa
29041 tgccctaggc ataaagtggt accctcactt tgcctgagaa ctgcaatctt caggaaaagt
29101 ggaaagaatt taccaaaccc tgaaatgcat cctcaataat ctctgtcagg aaatagcagt
29161 catgggtgga cctcttacct ttaaccctcc ttcaaatctg tattgcccct aaggctcccct
29221 agcaattaag ccccttttgag atcttatatt gtaggccatt tctatattca gacttatctg
29281 atactagatg aagaaactgc caaaatcacc cagtatgttt cttctttagc aggcttccaa
29341 cagactctct gggaatatgg gttaaaaaca aacccaacgt cggaagggga aacaatccca
29401 gcctctgtat cttccaggct ctagttctca ttaaagcttg gaaggttgaa accccaaatt
29461 cctaactaac tgtattctgg gggggccact tcactgctcc agtatctatt cccacagcca
29521 ttaaagtacc agagatagcc agctggatac atcatgcctg ggtgaagtca tggaaaggcc
29581 atgcaacacc agagccagaa ccaacaccct cagctccaga atacacttgt gaggcactgg
29641 aagacctgaa attcctcttt tagtgaaaac ataagtaatg ccccctcaac atcctttcac
29701 ctcaaagtaa ggtgatccta gtattaggaa ttttactaac aattgttatg attattatca
29761 ctattttaat cccaacttgt acaccaccag gagttccagt ggcagcttgc tttgtgatca
29821 atttctctct ctcacaatca ccttaggtca ccatggctct gctcctgttg gttctcatac
29881 tcaacgcttt actggcagca cactgccatc ctgatttcct gttatgagaa aaagctcagc
29941 aattgctcca aaacacagga tcccttact ccaccaaatg ctggttattt actagctctt
30001 cctctaaaac accagggaga gcttatccag cctgtaccag agattggaca agcatagaca
30061 tagaattacg tatttcctct caacaggacc ctaatctgaa agagctattt gggtctgcaa
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30181 tttggatcaa tcttttctaa tatcacttta atgggaataa cctctatttg tcttatggcc
30241 aaaagaaaaa gatggattgg atgtaggctc tttcccagta tagcttgtaa tgttactctc
30301 tctgtagatt ctaaccaaca gactgatgga acatacaccg aaaaccaatt ccaccatcaa
30361 ccaagattcc ccaaaccttc aaatattatc tttcttcagg caactttgct aattaagtcc
30421 attcagtttt gccagcaatg cccaagctca tgcagtactt gaaatttcta gttccagcct
30481 gatgattata accaatgtct gcaaattgcc aacctcagct ccacagcaga atgggttcta
30541 atggagtaaa ctcaaaattc tcttttttttg ggaaaatgaa accaagggag ctaatcagag
30601 ccaaacccca tgtacccaag tgttagcagg catgaatgta gctaccagct acctgggtgt
30661 gttgtcatcc ttggtatttt tgggggctgt cctcatctct tattttgttt tgacatctct
30721 acttgtctta aaacccaagg agccttctgt gtttgtggcc aatcagttta ccaatgcctc
30781 ctcattaaat ggactggaac ttgtaccata gattatgtac ccctggacat ctttatactc
30841 cctggcaatc tctctcttcc agcaccaatc catgggaatt ctatcttgcc cagggtgaaa
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30961 taaactgcct gaatctcaaa agcctccttg acctatagcc aactctgaaa ggaaatagcc
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31081 agttgtagtc ctccaaaatt gtcaaggact agatatgtta atggcagcac agggaggaat
31141 ttgtttagct ttagatgaaa aatgttgctt ttgggtaaat caatcagtaa aagtacaaaa
31201 caacatcaga caactcctaa atcgagcctc cagcttacaa gaacaagcct ctcgtggtta
31261 gttagattgg taaggaacct ggaaatggga tcttccctgg gttcttccct ttttaggccc
31321 acatgttagc ctctactttt gctcctttca gtccatgtct tcaaaaatct aataacccaa
31381 tttgtctcct ttctctcacc tttagatgat caagttccag atgatcctca gtgagggata
31441 ccatcctttc aatattcaag agtcacccctt ctacagagga ctcctagact tcccatcagt
31501 gggacatggc agaggtgaaa tcctgcccct gtctccctta gacctggctg gatactgctt
31561 ccaacaaccc atgcagccac cctgccctga cagctagcaa gaggccaaga cccacagaac
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31681 tcccaaagaa ttgggtcatg gatttttgcg ggggaaaatg ttagagtagg taattaggca
31741 gacatgagta agggaggaga gaccccctcc aactaggaat gtcaggtgag catcagatga
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31861 gaaagacaat ctcccaatag atagaaaagt cctgaagctg gtgatcagca gcttcctagt
31921 aagatctcag gatttgggca agcaggctca aacatgggca ctaagaggca aaatcgtgga
31981 gtttaactgg tatacagact tcctctaaaa acacatgact catgcacatg tggacagcct

FIG. 7-12

32041 gccccaagga aaaatcaaaa gaggagagat gcaaaacccc agaagcatgc caatatataa
32101 aaccccaagt ctaaggtcaa acagggcact tggatttctc aagttgccca cttggccctc
32161 ttctaagtgt actttatttc ctttcattcc tgctctaaaa ctttttaaat ttcactcctg
32221 ctcaaaaact tgcctcagtc tttcactctg ccttatgccc cttggaagaa ttatttcctt
32281 caagatggca aaaagcaagc tgctgcagac ccatacagat ttgctgctgc taacaggagg
32341 ataacttgag cccagaaggt taaggctgcg gtgagccttg attatgccac tgctctccag
32401 cctggcaaca gaatgagact ctattttttt ttcttttaaa aagacttttt acaaaaggca
32461 aaatgataat ggaactaaaa atgtacgttg actgacttaa ctccaactgc ttcttccctc
32521 aattgaaacc acctttgcaa aaattataac agtgagaaaa ttatggcagt aggggtgatc
32581 tgatcaagcc aaaccccatc ttgcctttag ccttcaagct gcccataatt attcctgggc
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32701 ttaaaattca accacctcag caaagctgat gagaggccac caggcaagga ggatagagga
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32821 aaatgtgcaa cttcttcaat tactcctgca gataacatca gtattttaga acctaagatt
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34381 ccaaattaaa cttaaagaag tttaattgag caatgaatga ttcacaaatc aggcagcctc
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35041 gtatatatta agtatagcag taaagcaaaa aaacaaaaaa aaacaagtat ttaacttttt
35101 tttggttttt tttttgaga cggagtctct ctctgttgcc caggctggaa tgcagtggca
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FIG. 7-13

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35341 ctcgcctcgg cctcccaaag tgctgggatt acaggcatga gccaccaagc ccggccttaa
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35461 ttaataggaa aatattaata atgagatgtt gactagcttt gtctgtctaa tgaaatttca
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35581 aatacacttg ctgaatacaa caattgaatt gtaaatagaa tagaaattat aaatgaactt
35641 ttcaatagta attattttt aatacgggta cttaaaattg tgtcaacttc ttaacaaaag
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35761 ggagacacgt cttcaagttg ctatgaatat aaactccaat tagcagcagt tacaagcggt
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37081 attttatgga ataccccata accgttcaca atttcttatt tcctagtttt cactagaaat
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37201 ataagggaga taactatatg caaagaaagt aagacatgtt tttggtaagg aaagccataa
37261 ggtataagga tgtgcttttt tttaaggaaa aggaagaata atttggtcta gtttggaggt
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37801 gaaaatgaca gttttctttt taaagagtat tgctccagtt tttaaaagtg attgtgaaaa
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38281 taattctagc actttgggag gccaaggtag gcagatcact tgagcctggg agtttgagac
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FIG. 7-14

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39061 acattgtcaa ttgcattata atgaactttc atcagatctt taaccatggc catttttaag
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40501 ccttaaggac ccttaaggag attttaagaa cctaatgaag aagagaattc acccaaaatt
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41101 agagattcat ggggcaaggt atgggggctc tttcttccat gccttctcca ggtgcaccat
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41281 gaacattatc tttactgcct ttcttccccc aacccccaga agtggaaagg ggtgctaaaa
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41401 ccaaacatat atttgcatga aatcatgaca tcctatagta atatttgatt ttcaaactct
41461 gcatgtataa tagttactta aaatttaaat taaattttaa aatgtaccag ttatgcagct
41521 gaattttggt actttttccc ttggctttgt ctggtatctg aatccaccat catttccctg
41581 tacaatatct gccattgga LI

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/13981

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : G01N 33/48, 33/53; C12Q 1/68
US CL : 436/64; 435/6, 975
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 436/64; 435/975

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, STN

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	TAKEUCHI, S. et al. Homozygous deletions at 9p21 in childhood acute lymphoblastic leukemia detected by microsatellite analysis. Leukemia. 1997, Vol. 11, pages 1636-1640, especially page 1636.	1-6, 18 and 22
Y	TAKITA, J. et al. Deletion Map of Chromosome 9 and p16 (CDKN2A) Gene Alterations in Neuroblastoma. Cancer Research. March 1997, Vol. 57, pages 907-912, especially page 907.	1-6, 10-18, and 22
Y	BATOVA, A. et al. Frequent and Selective Methylation of p15 and Deletion of Both p15 and p16 in T-Cell Acute Lymphoblastic Leukemia. Cancer Research. March 1997, Vol. 57, pages 832-236, especially page 832.	1-6, 10-18 and 22

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
13 SEPTEMBER 1999	19 OCT 1999

Name and mailing address of the ISA/US	Authorized officer
Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	Jennifer Hunt JOYCE BRIDGERS PARALEGAL SPECIALIST CHEMICAL MATRIX
Facsimile No. (703) 305-3230	Telephone No. (703) 308-0196

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/13981

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	SCHUYER, M et al. Sporadic CDKN2 (MTS1/p16 ^{ink4}) gene alterations in human ovarian tumours. British Journal of Cancer. 1996, Vol. 74, pages 1069-1073, especially page 1069.	1-6, 15, 18 and 22
Y	BATOVA, A. et al. Frequent Deletion in the Methylthioadenosine Phosphorylase Gene in T-Cell Acute Lymphoblastic Leukemia: Strategies for Enzyme-Targeted Therapy. Blood. 15 October 1996, Vol. 88, No. 8, pages 3083-3090, especially page 3083.	1-6, 11, 18 and 22
Y	TAKEUCHI, S. et al. Analysis of a Family of Cyclin-Dependent Kinase Inhibitors: p15/MTS2/INK4B, p16/MTS1/INK4A, and p18 Genes in Acute Lymphoblastic Leukemia of Childhood. Blood. 15 July 1995, Vol. 86, No. 2, pages 755-760, especially page 755.	1-6, 10-18, and 22
Y	STADLER, W. M. et al. Homozygous Deletions within Chromosomal Bands 9p21-22 in Bladder Cancer. Cancer Research. 15 April 1994, Vol. 54, pages 2060-2063, especially pages 2060.	1-6, 10-13, 16, 18, and 22
Y	MAO, L. Genetic Alterations as Clonal Markers for Bladder Cancer Detection in Urine. Journal of Cellular Biochemistry. 1996, Vol. 255, pages 191-196, especially page 191.	1-6, 16, 18, and 22
Y	HEYMAN, M. et al. Prognostic Importance of p15 ^{INK4B} and p16 ^{INK4} Gene Inactivation in Childhood Acute Lyphocytic Leukemia. Journal of Clinical Oncology. May 1996, Vol. 14, No. 5, pages 1512-1520, especially page 1512.	1-6, 11, 18, and 22
Y	LYDIATT, W. M. et al. Homozygous Deletions and Loss of Expression of the CDKN2 Gene Occur Frequently in Head and Neck Squamous Carcinoma Cell Lines But Infrequently in Primary Tumor. Genes, Chromosomes & Cancer. 1995, Vol. 13, pages 94-98, especially page 94.	1-6, 10-18, and 22
Y	US 5,739,027 A (KAMB) 14 April 1998, entire document.	1-6, 10-18, and 22
Y	US 5,714,329 A (DRACOPOLI et al.) 03 February 1998, entire document.	1-6, 10-18, and 22

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/13981

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 7-9 AND 19-21
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

Claims recite SEQ ID NOS which did not comply with sequence rules: no CRF or corresponding paper copy was provided.

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest
☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.